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OENOTHERA MUT. POLLICATA, AN INTERESTING NEW MUTATION[1]

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INTRODUCTION

MUTATION has been found to occur so consistently in every type of organism with which comprehensive genetical experiments have been made that the reporting of a single new mutation seems to be justified only on the ground of special considerations.

There are two such considerations which lead me to present the above-named form at this time. The great importance of having as many identified genes as possible, as an aid in the interpretation of the chromosomal mechanism of any given group of organisms, has long been recognized; but in the Oenotheras, wherein an unusually complex chromosomal mechanism is in the most urgent need of elucidation, the number of available gene mutations is still extremely small. Every new gene mutation found in this group of plants adds a new key for the solution of this difficult problem.

The second fact which seems to justify this report of *Oenothera* mut. *pollicata* is the type of the reaction which this new gene brings about. Genes have been found which affect in one way or another almost every kind of structure or function possessed by plants and animals. Be-

[1] Read before the General Section of the Botanical Society of America, at Cambridge, Mass., December 30, 1933. The experiments on which this paper is based have been supported in part by grants from the Bache Fund of the National Academy of Sciences and from the National Research Council.

cause many of the earlier Mendelian studies were made with such convenient characteristics as pigmentation, there was for a time an oft-repeated suggestion that genes have to do only with superficial traits.

Genes which produce structural modifications, of which many have been found, enter at once into the field of characteristics such as serve to differentiate the taxonomic groups and may well seem to be less superficial than the intensity or distribution of pigments. The genes which control the capsule form and the leaf-lobing in shepherd's-purse are of this nature, as are the genes of the short style and of double flowers in Oenothera. Such structural modifications teach that genes, which are undoubtedly in some sense chemical entities, are responsible for the number and direction of cell divisions which will take place within the specific region of effective activity of the given gene. In other words, a gene may determine the orientations of the successive mitotic spindles and the relative number of cell divisions which will take place in one direction as compared with the number in some other direction, within a given region or a given cell-lineage. The exact manner in which a gene produces this orientation of spindles and the limitation of number of divisions in any given branch of the cell lineage is not apparent, but that such specific action of certain genes is a reality can be shown by a study of the anatomical structure of any organ or organism whose shape is distinctly modified by the added presence of a single Mendelian gene.

DESCRIPTION OF THE NEW MUT. *Pollicata*

So far as present observations have gone the only recognized effects of the *pollicata* gene are to be found in the hypanthium and the gynaecium of the flower. As is well known, the hypanthium constitutes the most unique feature of the normal Oenothera flower, being a tubular structure at the proximal end of which is the ovary, while at the distal end the rim of this tube serves as a place for

the insertion of the sepals, petals and stamens. Through this tube the style extends as an independent organ from the top of the ovary to the stigmas. The length and stiffness of the style places the stigmas in a definite position characteristic of the particular species, this position in *Oenothera Lamarckiana* and other large-flowered species being pronouncedly distal to the anthers, thus strongly favoring cross-pollination.

In mut. *pollicata* the style originates as an independent structure, not from the top of the ovary, but from the distal end of the hypanthium (Fig. 1). The hypanthium

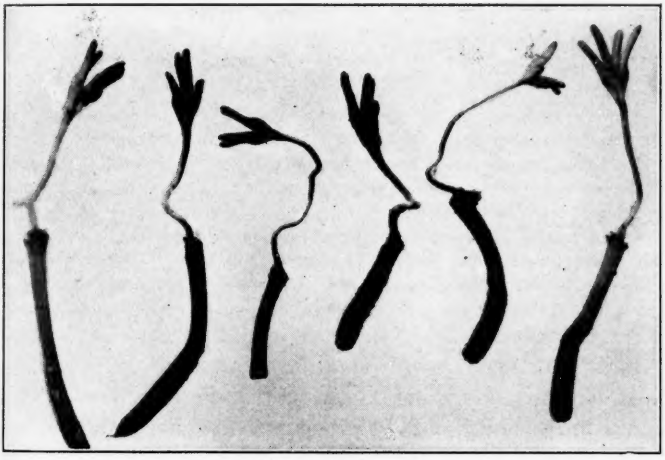

FIG. 1. Ovaries, hypanthia, styles and stigmas of six flowers of *Oenothera Lamarckiana* mut. *pollicata*, n. mut. ×1.27.

becomes a solid mass of tissue instead of a tube, but vestiges of the tube may remain as eight narrow air-passages lying in the radii which represent the positions of the median and marginal lines of the four sepals (compare figs. 2 and 3). Peripherally from these air-canals the structure of the hypanthium is not notably different from that of the normal flower, possessing both in the normal and in mut. *pollicata* a pair of rather large fibrovascular bundles radially arranged in the medial line of each sepal,

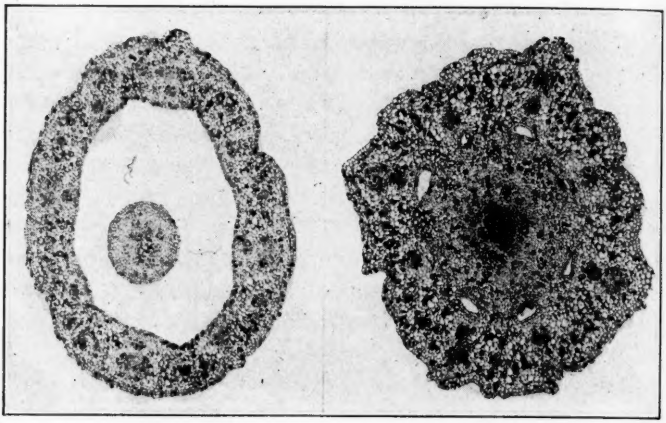

FIG. 2 FIG. 3

FIG. 2. Cross-section through the mid-region of the hypanthium of *Oenothera Lamarckiana*. Sections of hairs between the inner wall of the hypanthium and the style have been blocked out for the sake of added clearness. ×163. Microscopic preparation by W. H. Brittingham; photomicrograph by Fred Anderegg.

FIG. 3. Cross-section through the mid-region of the hypanthium of *Oe. Lamarckiana* mut. *pollicata* (Ped. No. 32277). ×163. Microscopic preparation by W. H. Brittingham; photomicrograph by Fred Anderegg.

a single large bundle in the marginal line between the two sepals, and three or four smaller bundles in each interval between these larger bundles.

Within the circle of the air-canals the change which results from the mut. *pollicata* gene is profound, for the cross-sections of the hypanthium in Figs. 2 and 3 show in the normal flower a continuous open space lined with a continuous epidermis, rather copiously clothed with hairs and containing a relatively thin style likewise bounded by a continuous epidermis which may also produce some trichomes, the hairiness of this region of the style being copious in the case of mut. *supplena*, but much less copious in the normal flower. Within the epidermis of the style in the normal flower is a cortex of thin-walled parenchyma surrounding a medulla of mostly smaller-celled parenchyma, and traversed near the cortex by four

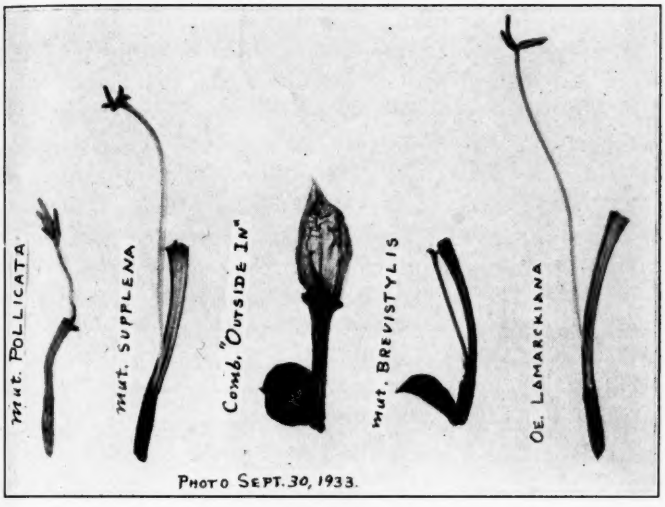

FIG. 4. Dissections of hypanthia of *Oenothera Lamarckiana*, and three of its mutants, *pollicata*, *supplena* and *brevistylis*. In each case, one side of the hypanthium has been sliced off down to the ovary, showing *pollicata* as a solid structure, the others as tubes containing the free styles. In the center is an "outside-in" flower, with one set each of sepals, petals and stamens removed, showing the replacement of the gynaecium by a large flower-bud containing, in repetition, all the floral organs except a gynaecium. This type is always produced by the interaction of the gene of mut. *brevistylis* with that of mut. *supplena*. The hypanthium of this was not sliced, since the gynaecium is obviously completely wanting. ×1.18.

very small bundles. In mut. *pollicata* the cortex of the stylar region has become enormously enlarged and is continuous with the ground tissue of the hypanthium wall, and instead of the four small vascular bundles of the normal style, the inner portion of the corticular region seems to have a rather disorganized group of numerous small bundles.

Although this solid hypanthium has lost the appearance of a style, it continues to function as one, for the pollen tubes make their way successfully to the ovules in the ovary at the base of the hypanthium and there effect fertilization.

At the distal end of the hypanthium the style arises as

a structure so devoid of mechanical elements that it can not support the weight of the stigmas, nor can it push the stigmas out beyond the stamens. Consequently, the stamens surround the stigmas when the pollen is shed and selfing takes place naturally, as it does in the mid-styled small-flowered species of Oenothera. The weakness of the proximal end of the style causes it to loop or kink as it grows in length. The thickness of the style increases distad from the thin proximal region and usually becomes noticeably heavy as the stigmas are approached. Here, instead of terminating in a neat circular disk out of which the thin stigmas grow and radiate at nearly right angles, as in the normal flower, no apparent disk is present, and the mostly clubby, awkward-appearing stigmatic lobes seem to have no very definite place of origin; they take a rather irregular more erect direction than in the normal flower. See Figs. 1 and 4.

Owing to the weakness of the proximal portion of the style and the heaviness of the stigmas, the position of the pistil in the open flower is determined by gravitation. It finds support on the lower side of the flower after the manner of the clapper in an upturned bell.

This description of the effect produced by the *pollicata* gene is based on the structures of *Oe. Lamarckiana,* with whose normal characteristic genes *pollicata* has been thus far associated. It will be interesting to observe any modifications which may be produced by association with other genes.

ORIGIN OF MUT. *Pollicata*

In the course of comprehensive studies of crossover phenomena in *Oenothera Lamarckiana,* a backcross of a trihybrid yellow single *Lamarckiana* having *rubricalyx* buds was made in 1929 with the completely green, old-gold double-flowered *pervirens.* Among the progeny from this cross (29218) both types of crossover—old-gold single and yellow double—were found, one of each in a family consisting otherwise of 44 old-gold double and 54

yellow single. In 1930 these two crossover individuals were crossed together, and since the two dominant traits were now in repulsion phase and each parent heterozygous the result (30303) was an apparent independence of the closely linked genes for single flowers *vs.* double, and yellow flowers *vs.* old-gold. The ratio in this family was 21 yellow single, 26 yellow double, 25 old-gold single and 14 old-gold double, the expected ratio being 21.5 of each of these types.

To show that, notwithstanding this apparent independence of these genes, they were nevertheless still closely linked, a single yellow from family 30303 was backcrossed in 1931 to old-gold double, and the progeny (31303) consisted of 36 yellow double, 34 old-gold single, 5 yellow single, 3 old-gold double, and 2 old-gold which showed no extra petals, but had a disturbance of the stigmas which led to their classification with the doubles. These two plants, which are now assumed to have been mut. *pollicata,* were only superficially examined and thus their profound modification from the normal structure was not observed. Since the old-gold plants in this family were generally either clearly single-flowered, or definitely double-flowered it was thought desirable to test the validity of the assumption that these two plants with modified stigmas were low-grade crossover doubles.

For this purpose a cross was made, using pollen of one of these two plants, 31303(46), on one of its double yellow sibs (45). The resulting progeny (32378), grown in 1933, consisted of but three plants—two old-gold single and one yellow double—but the result was a crucial proof that the unique plant 31303(46) was not a double, since in that case it could have produced only doubles in a cross with another double.

Whether the other plant 31303(98) which had been recorded in the same manner as (46) was identical with the latter genotypically can not be stated, since it was not tested, but I believe that both of these plants were mut. *pollicata.*

A culture (32277), produced by selfing 31303(46), developed rather slowly, and only 11 out of 95 reached the flowering stage. All were old-gold *pervirens* like the parent, and 10 were mut. *pollicata* and 1 double-flowered (mut. *supplena*). This indicates that mut. *pollicata* is a distinct and permanent hereditary form and that it is presumably dominant to *supplena*. In the family in which it was first found (31303) it should have been classed, therefore, with the old-gold singles as a non-crossover, not a low-grade double crossover, as originally supposed.

DISCUSSION

The addition of this new gene to the group of factors available for the "marking" of the chromosomes will owe much of its significance to the location of the *pollicata* gene with reference to other genes, to its uniformity of expression in different combinations, and to the cultural effectiveness of plants which possess it. Too little is known concerning these points to allow a safe prediction. That it is a gene mutation rather than the result of some form of chromosomal aberration is rendered certain by the fact that the mut. *pollicata* has the circle of twelve chromosomes and one pair characteristic of *Lamarckiana*, as kindly determined for me by Mr. W. H. Brittingham.

The evidence now available indicates that *pollicata* is epistatic to *supplena*, but its relation to the wild-type gene for single flowers is still an enigma, since the cross with a double-flowered sib produced single-flowered offspring, when it might perhaps have been expected to produce only *pollicata* and *supplena*. The reaction with *brevistylis* is also awaited with keen anticipation. Since the style of *brevistylis* is normally completely or nearly completely included within the hypanthium, will the formation of a solid hypanthium completely eliminate the style, leaving an isolated ovary at the base with only *a stigmatic spot* at the top of the pedicel-like hypanthium, or will the gynaecium be completely eliminated as it is in

the "outside-in" flower which results from the interaction of *supplena* and *brevistylis*.[2]

The slow development of this first culture of mut. *pollicata* is somewhat discouraging, but perhaps this is not a necessary association, and the possibility of developing a more precocious strain must be studied. Most of the recessive gene-mutations thus far found in Oenothera seem to affect speed of development disadvantageously. Only the *brevistylis* gene seems to have a somewhat accelerating effect, while *pervirens* apparently has no material effect on speed of development, but has the very great advantage of giving a higher percentage germination of the seeds than in *Lamarckiana*.

While the evidence for the relative location of *pollicata* among the known genes is still very meager, it seems likely from the occurrence of two individuals in the original culture that they represented a segregation from a heterozygous parent, rather than the direct result of the mutational process. If this assumption is correct, the *pollicata* gene should be found in the first linkage group, since any other position should have given a larger proportion of *pollicata* plants in the original culture. A confirmation of this suggestion awaits the result of experimentation which has been started with this gene.

The occurrence of this mutation, which so profoundly modifies the gynaecium, in connection with the previously discovered genes of mut. *brevistylis* and mut. *supplena*, hints that the gynaecium of Oenothera is peculiarly subject to mutational modification. This discovery is in keeping with the fact that so many of the numerous species of Oenothera have notably unique differences in length of style and in shape and size of the stigmatic lobes. Dissection views of the hypanthium and style of mutants *pollicata*, *brevistylis* and *supplena*, in comparison with the parent *Oe. Lamarckiana* and with the "out-

[2] G. H. Shull, "The 'Outside-in' Oenothera Flower, a New Morphological Type Produced by the Interaction of Two Recessive Mendelian Factors," *Proc. Nat. Acad. Sci.* 14: 142–146, 1928.

side-in'' type produced by the interaction of *brevistylis* and *supplena* genes, are shown in Fig. 4. Outside-in has completely lost its ovary, and the hypanthium is here replaced by a solid pedicel. In some of its manifestations the *supplena* gene by itself eliminates the ovary and renders the plant incapable of being used as a seed-parent, though generally having abundance of pollen.

SUMMARY

(1) *Oenothera* mut. *pollicata,* first found in 1932, greatly modifies the hypanthium and the style and stigmas, converting the hypanthium into a solid structure, and adapting the flower to self-fertilization by keeping the stigmas surrounded by the stamens at the time the pollen is shed.

(2) The morphology and anatomy of the new type are described and illustrated.

(3) The first progeny from selfing one of the two original plants consisted of ten *pollicata* and one *supplena.*

(4) A cross with a double-flowered *supplena* sib produced a progeny containing no *pollicata,* but single-flowered and double-flowered plants.

(5) That *pollicata* is a gene mutation is made certain, not only by the specific character of the modification it produces, but also by the fact that the chromosome situation of the parent type remains unchanged, the *pollicata* plants having the circle of twelve and one pair of chromosomes characteristic of *Lamarckiana.*

(6) Mut. *pollicata* is probably in the first linkage group.

THE ARTIFICIAL PRODUCTION OF MUTATIONS BY ULTRA-VIOLET LIGHT

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THE now classical discovery by Muller that mutations and rearrangements of genes are produced by x-rays was followed by the demonstration, by Muller and Mott-Smith, that despite their efficacy, x-rays can be responsible for only a part of the mutations that occur in nature. The only other agent hitherto known to alter genes is heat. The first experiments with heat (Muller and Altenburg) indicated that there is a doubling of the mutation rate for a 10° increase in temperature, and this was subsequently confirmed in a series of extensive experiments by Muller (*Genetics,* 1925). Extreme heat has been shown to cause a more marked increase in mutation rate (Jollos, Goldschmidt, Plough, etc.). Since none of these effects is sufficient to explain completely the natural rate of mutations, other agents must be operative. The work reported in the present paper demonstrates definitely that mutations can be produced in abundance by ultra-violet light.

Three series of experiments were performed; one series on the adults, a second on the larvae and a third on the eggs. The source of the ultra-violet light was a quartz mercury arc lamp. The distance of the flies from the lamp varied in different experiments, but on the average it was about 1 meter; and usually the lamp was run at about 50 volts. In most of the experiments, eosin or yellow eosin males were treated and the X-chromosome was examined for lethals by Muller's Cl B method. Treated and control flies as a rule were derived from the same stock bottle, in a given experiment, and both lots were given the same treatment, except that the controls were protected from the ultra-violet light by a piece of plate glass. This absorbed practically all the ultra-violet but little or none of the other radiation coming from the

lamp, so that the only difference between treated and controls was in the amount of ultra-violet light they received.

During the course of these experiments a total of over 50,000 families were reared and examined for lethals. A total of 98 lethals were found, all of independent origin; of these 79 were in the treated families, 19 in the controls. Including lethals not of independent origin, the total was 177. The location in the X-chromosome of all lethals was determined (with the exception of two lethals that were lost before they were located). The lethals were located by selecting the round-eyed females in the F_2 of composition $\frac{w^e(\text{treated})}{v\ f}$, and making a count of their sons (over 100 as a rule). Thus each lethal was located with regard to the w^e, v, f-loci. If a female, of the above composition, produced + sons but no $w^e f$, then the lethal was located between w^e and v. If she produced $w^e f$, but no + sons, then the lethal was located between the v and f-loci. Since the w^e and f-loci are approximately at the two ends of the X-chromosome, practically the entire length of the genetic chromosome was covered. For the sake of convenience, we can label the region between w^e and v "I," and between v and f "II." These regions are approximately equal in length.

In case two lethals came from the same P_1 male, they might have been derived through multiplication from the same original lethal, especially if they were found by linkage tests to be located in the same part of the X-chromosome. We can therefore not definitely count two lethals as of independent origin if they come from the same male and are found to be located in the same part of the X-chromosome. But since some of the lethals occurring in the same region of the chromosome were probably at different loci, the number of lethals regarded as of independent origin is the minimum number.

In the tables given below, the number of lethals that belong together is indicated by an exponent. Thus, II^2 means two lethals in region II derived from the same male. However, if the two lethals in region II came from

different males in a given experiment, they are counted as two separate lethals and indicated as 2 II. Some of the mutations were semi-lethals. These are indicated by the superscript "s." Thus, $(II^s)^2$ would mean a semi-lethal in region II, repeated twice. If the semi-lethal was also a visible mutation, it was indicated by a superscript "v"; thus, $(II^{sv})^2$.

None of 79 lethals found in the treated lot was accompanied by disturbances of crossing-over. Ultra-violet light therefore produces no inversions or other chromosomal changes that are detectable from changed cross-over values. In this respect, the effect of ultra-violet light differs markedly from that of x-rays.

Visible mutations were not looked for, but three were noticed in the treated lot and none in the controls. Of the three in question, two were wing changes and were sex-linked, the third was an eye color change and was autosomal. One of the wing mutations was a small, antler-like wing bent forward at right angles to the body and curved down. It was a semi-lethal. It was very definite when it first appeared in the F_2, and all the males that came through had it (11 males in four vials all derived from the same treated male). But it disappeared completely in the F_3, the wing appearing normal in the few males that developed (four). The change in the visible effect of the mutation may have been due simply to changed cultural conditions (the F_2 were grown in vials, the F_3 in bottles). On the other hand, the change may have been due to an instability of the mutated gene for a short period after the original mutation. This matter requires further investigation.

Experiments in Which Adults Were Treated

The experiments on the adults were long drawn out (extending over a period of five years) because of difficulties connected with the treatment. Ultra-violet light does not penetrate very deeply below the superficial tissues, at least not in any great amount. Moreover, it has a highly destructive action on tissues in general and

can be used only in very limited dosages without killing the flies. The effect of the treatment on the adults was therefore only very slight, and it became necessary to conduct the experiment on a very large scale in order to get a statistically significant result. It was thought that the high screening effect of the superficial tissues might be partly eliminated by the use of eosin or of yellow eosin stocks. Both eosin and yellow have less pigment than the normal fly in the skin and in the testicular envelopes. Therefore these stocks were used for treatment.

Table 1 gives the data in one set of experiments involving the treatment of the adults.

TABLE 1

ADULTS TREATED (y w^e)

Exp. no.	Treatment			Count of F_1			
	Time (hours)	Distance (meters)	Volts	Treated		Controls	
				No. bred	Lethals	No. bred	Lethals
14	97	3	40	238	.....	73	.....
15	134½	4.3	40	78	.....	84	.....
16	28	1	40?	154	1(I)	136	.....
17	26	1	40?	57	.....	388	.....
19	28	1	40?	484	1(I) (II)	769	.....
26	19	1	40	1825	1(I) (II)	1,988	.....
27	19¾	1	40	1547	.....	1,414	.....
28	20	1	40?	68	.....	69	.....
30	24	1	50	797	1(I) (II)	659	.....
31	22½	1	40	218	.....	277	.....
35	23½	1	40	1,295	5(I)	1,449	2(I)
			Totals	**6,761**	**13**	**7,306**	**2**

In one set of three experiments, separate controls were not run for each experiment. Instead, one set of controls was run for the three, concurrently with them. The data for this set of experiments are given in Table 2.

In another set of experiments, the controls were lost (because of mold in the F_2 vials). Another lot of flies from the same stock was therefore examined later.

TABLE 2

ADULTS TREATED (WILD). ONE SET OF CONTROLS

Experiment no.	Treatment (in hours)	Distance (in meters)	No. of F_1 bred	Lethals
18	33–53	2	98	.....
19	7½–24	2	341	.....
20	11–36	2	141	.....
	Totals for treated		580	.....
	Controls		375	.....

These, of course, were not controls in the strict sense; but they may be considered as approximate controls, since they were derived from the same stock. The data for this set of experiments are given in Table 3.

In an earlier set of experiments the lethals were detected not by the C1 B method but by linkage counts.

TABLE 3

ADULTS TREATED (y w and y w^e). "CONTROLS" RUN ABOUT A MONTH AFTER THE TREATED, BUT DERIVED FROM THE SAME STOCKS AS THE TREATED

Exp. no.	Char. of P_1 ♂'s	Treatment			F_1 from treated	
		Time (in hrs.)	Distance (in meters)	Volts	No. bred	Lethals
23a	y w	½	3	60	67	1(I)[2]
23b	y w^e	½	3	60	38	.....
24a	y w	1½	3	60	30	.....
24b	y w^e	1½	3	60	104	.....
25a	y w	2	3	60	110	.....
25b	y w^e	2	3	60	79	1(II^s)
26a	y w	4½	3	60	89	1(II)
26b	y w^e	4½	3	60	79	.....
30	y w	41½	2–3	60	15	.....
34	y w	11½	3	60–90	24	.....
36	y w	24¾	3	60–90	81	1(I) 1(II)[2]
37	y w	22	3	60–90	230	1(I^s)
38	y w	44	3	60–90	98	1(II)
39b	y w	23	3	90	49	1
40b	y w	46	3	90	6	.....
	Totals				1,099	8
	Controls (y w and y w^e)				214	.....

The data for these experiments have already been published (Altenburg, 1928); but they are given in Table 4 for the sake of completeness.

TABLE 4

ADULTS TREATED*

Exp. no.	Treatment (duration)	Count	Lethals in treated	Lethals in control
1	1 hr. 10 min.	131	1(I) 1(II)	.....
2	1 hr.	49	.....	.....
3	1 hr.	11	.....	.....
4	30 min.	10	.....	.....
5	45 min.	66	.....	.....
6	30 min.	64	.....	.....
7	30 min.	60	.....	.....
8	1 hr. 50 min.	81	.....	.....
9	1 hr. 50 min.	103	1(I)	.....
10	4 hrs.	56	.....	.....
11	4 hrs.	49	.....	.....
12	4 hrs.	84	.....	.....
13	4 hrs.	35	.....	.....
Totals		799	3	.....

* The P_1 were bb ♂♂ (treated) × sc v f ♀♀. The sc v f chromosome in the $F_1 \frac{bb}{sc\ v\ f}$ ♀'s served as a control for the treated (bb) chromosome. Distance of flies from lamp, 5 cm. (Previously reported in the AMERICAN NATURALIST, 62: 540–545, 1928.)

The totals for all the "adult treated" experiments are as follows:

Treated lot	24 mutations in 9,239
Controls	2 mutations in 8,694

The difference in the lethal rate in treated and controls is significant. Apparently, therefore, occasional rays of the ultra-violet light penetrate the skin of the adults and reach the reproductive cells.

EXPERIMENTS IN WHICH LARVAE WERE TREATED

In the second series of experiments the ultra-violet light was applied to the larvae instead of the adults. It

was hoped that larvae, being more transparent to ordinary light, might also be more transparent to ultra-violet. The intensity of the light was decreased by increasing the distance of the larvae from the lamp; and thus the treatment was prolonged over several days. The larvae were allowed to develop on the food for 2 or 3 days before the treatment was started, because it was found that eggs treated immediately after being laid were killed and never hatched into larvae. The parents were included with the larvae in the treatment in the hope that the eggs of the parents might be affected. It was learned later, however, that all eggs are killed at a certain susceptible period of their development; hence the only individuals that survived the treatment were those which had developed as larvae for 2 days (or somewhat less) before treatment began. The data for the "larvae treated" experiments are given in Table 5.

TABLE 5

LARVAE TREATED (y w^e STOCKS)

Exp. no.	Treatment			Count of F_1			
				Treated		Controls	
	Time (days)	Distance (meters)	Volts	No. bred	Lethals	No. bred	Lethals
9	5	3	40	502	1(I)	296	
10	7	4.3	40	178		147	1(I)
11	7	4.3	40	135	1(II)		
13	5	3	40	63			
14	5	3	40	44		108	
15	4	4.3	40	139		148	
19	4¾	1	?	1554	1(I)[2], 1(II)[2]	1784	
I–IV	?	?	?	212		176	
			Totals	2,827	4	2,659	1

In another set of experiments, the treatment was continued on the adults that developed from the treated larvae. The data for these experiments are given in Table 6.

TABLE 6

TREATMENT APPLIED TO LARVAE AND TO ADULTS DERIVED FROM THE TREATED LARVAE (y w STOCKS)

Exp. no.	Treatment: Time (in hrs.)	Distance (meters)	Volts	Count of F_1: Treated No. bred	Treated Lethals	Controls No. bred	Controls Lethals
41L4	larvae 92 / adults 24	4	60–90	109	1(I)	158	.....
41L5	larvae 92½ / adults 48	4	60–90	162	1(I?) 1(II)	277	.....
	Totals			271	3	435	.....

The totals for the experiments in which the larvae were treated are as follows:

Treated lot	7 mutations in 3,098
Controls	1 mutation in 3,094

The difference in the treated and controls again seems significant.

The tissues of the larvae are apparently just about as impervious to the ultra-violet light as the tissues of the adult if we may judge from the fact that the increases in mutation rate are of the same order of magnitude. This relative impermeability of the larvae was confirmed by direct measurements made for me with a Geiger counter by Dr. Gordon Locker. It is also shown by the fact that the larvae can stand just about as much of the ultra-violet light as the adults. Their resistance to the rays is due to the high screening effect of the superficial tissues. But at a certain stage in the development of the eggs, the superficial tissues are relatively transparent to the rays, and at this stage the developing flies are killed by less than 2 minutes' treatment (with the light at a distance of 1 meter and 60 volts). Thus it follows that the living cells are easily killed by ultra-violet light and that they are ordinarily protected by the superficial tissues. However, again as in the case of the adults, occasional rays seem to reach the germ cells in the larvae, and to produce mutations.

Experiments in Which the Eggs Were Treated

In the third set of experiments the treatment was applied to the "pole cells" of the developing eggs. It will be recalled that the pole cells are the germ tract cells, and that they appear at the amicropilar end of the developing egg 2¼ hours after fertilization, when the egg develops at 24° C. The pole cells are particularly advantageous for treatment with ultra-violet light, because they are very close to the surface of the egg and thus readily accessible to the rays. Moreover, in case a lethal is induced in a pole cell, it can definitely be identified as having arisen at the polar cap stage. For the lethal would be transmitted by division to all the unreduced cells descended from the polar cap cell; that is, it would be multiplied or "reduplicated" to just the extent that the polar cap cell is multiplied in the course of development. Therefore in order to find lethals that arise at the polar cap stage, I tested a fairly large number of sperm cells from a given male (that was treated in the egg stage), and looked for lethals that are reduplicated to the right amount. If a mutation occurred in one of the polar cap cells at the time that there were, say 10 cells in the polar cap, then 1/10 of the unreduced germ cells would receive the mutation. If the mutation occurred when there were 20 polar cap cells, then 1/20, or 5 per cent., of the unreduced germ cells would receive the mutation. For the reduced germ cells, the proportion containing autosomal mutations would be one half of those for the unreduced cells. But in the present experiments only the X-chromosome of the male was followed; and since this is transmitted to all the daughters, and since only the daughters were tested, the proportion of mutations in the reduced cells is the same as in the unreduced.

The polar cap lasts for about an hour (at 24° C.). The cells then migrate forward a little distance to a pocket in the dorsal surface, into which they sink. At the time that the polar cap migrates, it contains about 20 cells.

Thus, any mutation that is reduplicated in 1/20 or more of the reproductive cells may be regarded as having occurred originally in one of the germ cells at the polar cap stage.

After the germ tract cells have got past the polar cap stage, they become more numerous and each one gives rise to a correspondingly smaller proportion of the reproductive cells of the adult. Accordingly, a mutation that occurred at a later stage would not be reduplicated to any great extent and would appear as an "isolated" mutation. Most of the "natural" mutations would occur after the polar cap stage, because the cells are now more numerous, and further because the mutations have more time in which to occur. The natural mutations would therefore appear as isolated mutations. Thus, if the treatment is given at the polar cap stage, we can distinguish between the "induced" and the "natural" mutations from the relative extent to which they are reduplicated.

On *a priori* grounds, one would seldom expect a lethal mutation to occur at just the polar cap stage in untreated material; first because there are so few cells present at this stage, and second because the cells do not remain long in the polar cap stage (only about an hour). It would therefore be very unusual if a mutation took place at just the polar cap stage (one chance in 28,800, if the natural mutation rate is put at one sex-linked lethal per 800 cells, per 15-day reproductive cycle, and further if we consider that there are 10 cells in the polar cap on the average, and that the polar cap lasts for one hour). Consequently, if a lethal did turn up in material treated at the polar cap stage, one could with a fair degree of certainty ascribe the occurrence of the lethal to the treatment. In other words, we have here a method of identifying a particular lethal with the treatment. The relationship of cause and effect is almost as evident as when we press a push button and get the sound of a bell. Therefore the very large counts that are necessary with

the ordinary method are no longer required. A relatively few reduplicated lethals will suffice to demonstrate an effect of the agent.

Geigy was first to treat the polar cap cells with ultra-violet light. However, Geigy did not arrange his experiments in such a way as to detect reduplications, and so his results were inconclusive.

In the "egg" experiments, only the polar cap ends of the egg were treated. The rest of the egg was shielded from the ultra-violet rays. One method of shielding the eggs was to place a cover glass horizontally on some food, and then to bury the eggs, polar cap end up, along the edge of the slide, so that only the polar caps projected above the level of the cover glass, like the heads of soldiers in a trench. To carry the analogy further, the ultra-violet rays corresponded in position and direction to the gunfire. A second method of shielding the eggs was simply to place them along the sharply cut edge of a moist piece of blotting paper, with only the polar cap ends projecting beyond the edge. The blotting paper was then set on edge (properly supported). Thus the polar cap ends of the eggs projected above the edge of the blotting paper like the heads of people above a high fence. The ultra-violet light corresponded in position and direction to the rays of the setting sun, beyond the fence.

Two sets of egg experiments, an earlier and later, were performed. The later set will be described first, because they give the results in a somewhat more clear-cut manner than those done at the earlier period when I had not yet been able to get large numbers of eggs all in the polar cap stage at the same time. In the second set of experiments, I got the females to lay their eggs rapidly, and thus got all the eggs, or almost all, in the same stage of development. Moreover, at the suggestion of Miss Frances Ward, the living eggs were examined just before treatment, as an additional precaution that they were at the right stage.

The results of the second set of egg experiments are

as follows. Among 117 eggs treated in the polar cap stage, there were 7 lethals that were reduplicated to the extent of 5 per cent. or more of the total reproductive cells. Among 114 control eggs, there were no lethals reduplicated to the extent of 5 per cent.

In these egg experiments, lethals that are reduplicated to the extent of 5 per cent. or more of the total reproductive cells are regarded as having arisen in the polar cap stage, as above mentioned. Accordingly, there must have been 7 lethals that arose at the polar cap stage in the treated lot, and none in the controls. This result demonstrates that ultra-violet light produces mutations. If we put the results in terms of cells and consider that at the time of treatment there are on the average 10 cells per polar cap, and 117 treated polar caps, then about one cell in 167 mutated in the polar cap stage in the treated lot, but none in the controls. It should be borne in mind that the polar cap stage lasts for only about 1 hour, and that 1 mutation per 167 cells per hour is a much higher rate of mutation than the natural rate. It is actually over 1,700 times as high, if we consider the natural rate about 1 cell in 800 over the 15-day cycle of the fly as determined in the controls of the egg experiments.

In both the treated and controls, in the above egg experiment, "isolated" lethals arose; that is, lethals that were not reduplicated. Most of these isolated lethals, in the treated material, were not due to the treatment; for not being reduplicated, they did not arise at the polar cap stage; that is, at the time of treatment. They therefore represent "natural" lethals. Some of the isolated lethals may, however, have been due to mutations induced in eggs that were beyond the polar cap stage (a certain number of such eggs being unavoidable). Thus the proportion of isolated lethals in the treated individuals should be higher than in the controls. This is actually found to be the case. The figures are as follows. Among the 117 treated eggs, there were 18 isolated lethals; among the 114 controls, there were 7 isolated lethals.

The figures for the earlier series of egg experiments

are as follows. Among 122 treated males, there were 6 reduplicated lethals. Among 108 controls, there was 1 reduplicated lethal.

As previously mentioned, a mutation is not very likely to occur at just the polar cap stage in untreated material. It is possible that some stray ultra-violet light, reflected from the walls of the room, got around the sides of the plate glass which was placed in front of the controls in the first set of egg experiments, and thus one of the eggs may have been affected. In the second set of egg experiments, the sides of the controls were protected to prevent stray ultra-violet light from striking them.

The number of "isolated" lethals in this first series of egg experiments was also greater in the treated than in the controls. The figures are as follows. Among the 122 treated males, there were 14 isolated lethals. Among the 108 controls, there were 6 isolated lethals. This difference may have been due to chance, but as the results are in the same direction as in the other series of egg experiments, it seems more likely that the difference in question was due to the treatment of eggs that were beyond the polar cap stage.

Most of the egg experiments were done with unshelled eggs. In some, however, shelled eggs were used. These did not yield any higher proportion of lethals than did the unshelled material.

In the tests for lethals in the second series of egg experiments, as a rule 50 to 60 daughters were bred from each male that had hatched from the treated and control eggs. When this number of daughters are bred, the chances are comparatively small that a reduplicated lethal will fail entirely to be detected (at least once), provided the lethal is reduplicated to the extent of 5 per cent. or more of the total reproductive cells. According to the calculations of Dr. Hewitt Dix, of the mathematics department of the Rice Institute, the chances are on the average only 1 in 16 that a lethal will not show up at all, if 54 daughters are bred, and if the lethal is reduplicated to the extent of 5 per cent. of the reproductive cells.

In these later egg experiments, usually all the daughters of both the treated and control males were saved in addition to the 50 to 60 that were bred. If a lethal did turn up among the 50 to 60 already bred, the extra females that had been saved were bred in order to ascertain whether the lethal continued to be reduplicated to the same extent as in the original tested lot. If the lethal appeared to be an "isolated" lethal in the original lot, this was confirmed or disproved by breeding the extra females.

The detailed data in connection with the egg experiments are given in Tables 7 and 8 below. In these tables, the Roman numeral indicates as in other tables the location of the lethal. The figures following this numeral indicate the number of times the lethal was found and in how large a count. Thus, I.18.51 would mean that from a given egg a lethal was obtained in region I and that this lethal was reduplicated 18 times in a count of 51. In one or two cases, the lethal was so close to vermilion that I could not tell on which side it was. These lethals are indicated by a "v" in place of the Roman numeral. In one case, a lethal was lost before it was located; this is indicated by a question mark in place of the Roman numeral. These tables do not give the number of F_1 daughters bred from each treated male, as this would expand the tables too much. But the total number of daughters bred in each experiment is given.

Outstanding cases of reduplication are 18 lethals in a count of 51, seven in a count of 77, nine in a count of 104 and 14 in a count of 149.

The totals for both sets of egg experiments are given in Table 9.

The results of both sets of egg experiments quite definitely establish an effect of ultra-violet light on the mutation rate as shown by the almost exclusive occurrence of lethals, reduplicated over 5 per cent., in the treated lots. Moreover, the results of the earlier experiments, on the adults and larvae, confirm the conclusion that there is such an effect.

TABLE 7

POLAR CAP OF EGGS TREATED. EOSIN STOCKS USED. DISTANCE FROM LAMP 150 CM. LAMP RUN AT 50 VOLTS. FALL, 1933 (SECOND SERIES OF EGG EXPERIMENTS)

Exp. no.	Duration of treatment	Treated						Controls					
				Lethals						Lethals			
		No. eggs treated	No. F_1 ♀'s bred	Redup. 5 per cent. or over	Redup. below 5 per cent.	Isolated	Total	No. of eggs	No. F_1 ♀'s bred	Redup. 5 per cent. or over	Redup. below 5 per cent.	Isolated	Total
1	2 min. 50 sec.	2	98	.....	.....	II^s.1.82	1	2	156	.....	.....	I.1.82	1
2	3 min.	6	256	.....	.....	.....	.....	5	387	.....	.....	II.1.41	1
5	3 min.	6	457	.....	.....	.....	.....	8	383	.....	.....	.....	.....
8	2½ min.	7	286	I.18.51	.....	.....	1	6	314	.....	.....	II.1.70	1
10	1 min.	11	741	I.6.108	II.3.104	I.1.110	3	10	582	.....	II.2.101	.....	1
11	1 min.	2	71	.....	.....	.....	.....	2	105	.....	.....	.....	.....
13	1 min.	19	836	I^{sv}.4.59 I.2.16	.....	I.1.51 II^s.1.51	4	19	1,043	.....	.....	II.1.81 I.1.49	2
14	1½ min.	12	761	I^s.6.107 II.9.104	.....	I.1.76	3	10	531	.....	.....	.....	.....
15*	20 sec.	8	402	.....	.....	II.1.67	1	8	408	.....	.....	.....	.....
19	1 min. 20 sec.	16	877	II.7.77	1.2.48	v.1.44 II.1.81 II.1.74 ?.1.61	6	17	855	.....	.....	.....	.....
20	1½ min.	9	353	.....	.....	II.1.49 I.1.14	2	9	521	.....	.....	II.1.98	1
22*	30 sec.	7	379	.....	.....	I.1.40 v.1.40 II.1.56	3	7	383	.....	.....	.....	.....
23*	22 sec.	12	685	.....	.....	I.1.59 I^s.1.57 I.1.64	3	11	774	.....	.....	I^s.1.54	1
Totals		117	6,202	7	2	18	27	114	6,442	.....	1	7	8

* Shelled eggs.

TABLE 8

POLAR CAP OF EGGS TREATED. WILD TYPE STOCKS (SUPPLIED BY TEXAS UNIVERSITY, ZOOLOGY DEPT.) USED IN THE FIRST SEVEN EXPERIMENTS, EOSIN IN THE REST. DISTANCE FROM LAMP 150 CM. LAMP RUN AT 50 VOLTS. SPRING 1933 (EARLIER EGG EXPERIMENTS)

		Treated						Controls					
				Lethals						Lethals			
Exp. no.	Duration of treat. (in min.)	No. eggs treated	No. F_1 ♀'s bred	Redup. 5 per cent. or over	Redup. below 5 per cent.	Isolated	Total	No. of eggs	No. F_1 ♀'s bred	Redup. 5 per cent. or over	Redup. below 5 per cent.	Isolated	Total
10	14	4	706	.....	.....	.....	.....	3	369	.....	.....	I.1.166	1
11	9	3	151	.....	.....	.....	.....	3	154	.....	.....	.....	.....
12	7	3	91	.....	.....	I.1.35	1	3	83	.....	.....	I.1.17	1
13	7	4	184	.....	.....	I.1.31	1	5	180	.....	.....	.....	.....
15	10	8	300	.....	.....	I.1.82	1	3	86	.....	.....	.....	.....
19	5	18	726	I.2.24	.....	II.1.52	2	19	858	.....	.....	.....	.....
20	6	1	63	.....	.....	.....	.....	2	75	.....	.....	.....	.....
24	8	3	93	.....	.....	.....	.....	3	127	.....	.....	I.1.55	1
25	5	1	125	.....	.....	.....	.....	1	101	.....	.....	.....	.....
27	5	3	261	.....	.....	II.1.103	1	3	176	.....	.....	.....	.....
28	5	7	292	.....	.....	II^{sv}.1.51	1	5	441	.....	.....	.....	.....
29	4	1	91	.....	.....	.....	.....	1	95	.....	.....	.....	.....
31	3	22	2255	.....	II.2.43	II.1.157	2	24	1875	.....	II.2.58	I.1.157 II^{s}.1.120 II.1.152	4
32	4	12	1029	1.4.99	.....	II.1.125 II.1.159 I.1.139 II.1.139	5	10	708	.....	.....	.....	.....
33	4	3	156	.....	.....	II.1.51	1	3	116	.....	.....	.....	.....
34	4	11	660	II.14.149 I.3.35 I^{s}.2.36	.....	.....	3	7	547	.....	.....	.....	.....
36	2	18	674	II.6.39	.....	I.1.23 I.1.13	3	13	630	v.5.70	.....	.....	1
Totals		122	7,857	6	1	14	21	108	6,621	1	1	6	8

TABLE 9

TOTAL COUNTS FOR EXPERIMENTS ON TREATMENT OF EGGS

	No. eggs	No. F_1 ♀'s bred	Lethals			
			Redup. 5 per cent. or more	Redup. below 5 per cent.	Isolated	Total
Treated	239	14,059	13	3	32	48
Controls	222	13,063	1	2	13	16

I wish to thank the American Academy of Arts and Sciences for a grant of $500 to defray the expenses of an assistant during the year 1931–32. To Miss Frances Floed and Miss Frances Ward I owe many thanks for their capable assistance and interest in the work. I wish also to thank Dr. Lewis Mott-Smith, of the physics department of the Rice Institute, for much help in the setting up of the physical apparatus, Dr. Gordon Locker for making the Geiger counter measurements, and Dr. Hewitt Dix for help in calculating probable errors.

SUMMARY

Ultra-violet light produces mutations. This is especially evident if the developing eggs are treated at the polar cap stage, at which time the germ tract cells are close to the surface of the egg and readily accessible to the ultra-violet light. The effect is clearly established by the method of "reduplicated mutations," whereby a mutation, if induced at the polar cap stage, can be definitely identified through the multiplication or "reduplication" of the mutant gene in the germ tract during the course of development.

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THE PRODUCTION OF STERILITY IN *DROSOPHILA MELANOGASTER* BY X-RAY TREATMENT

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INTRODUCTION

IT has been known for several years that x-rays, when applied to germinal tissues, produce sterile individuals. Muller (1928) reported a high percentage of sterility among both males and females of *Drosophila* following exposure of adults to x-ray emanations. Subsequently, Hanson (1928) observed a high degree of sterility among males of *Drosophila melanogaster* to which x-ray treatment had been administered and a publication by Hanson and Ferris (1929) makes note of the sterility produced by the exposure of adult banana flies to irradiations.

These observations apply only to cases in which germinal tissues of adults were exposed to irradiations. In 1932, the writer published a brief note on the comparative sterility results obtained by treating germ-cells of *Drosophila* in different stages of development. The present paper lends support to the published results, introduces a new phase of sterility induced by x-rays and offers a plausible explanation for its production.

MATERIAL AND METHODS

The material employed for experimental purposes consisted of adults and larvae of *Drosophila melanogaster*. Adults, both male and female, were irradiated immediately after emergence from the puparium and before fertilization of the females had been accomplished. Eggs were collected and a portion of the developing organisms treated with x-rays at the age of 35–36 hours while another portion received treatment of equal intensity when the embryos had attained the age of 71–72 hours. Males and females from cultures irradiated in the embryonic

stages were separated before copulation occurred and were mated as in the case of flies treated as imagoes.

Treated virgin females and virgins from cultures irradiated as larvae were mated individually to untreated males (two or more males being used in each case) while all males were mated individually, two or more untreated females being employed in each instance. Matings were made in vials and sterility determined by the absence of offspring in the cultures.

The treatment administered to all groups was of equal intensity—1325 *r* units. Conditions of temperature and food remained constant throughout the experiment, the temperature being maintained at 27° C. The one variable in the entire procedure was the age at which the organisms were irradiated.

It had been noticed in previous work that variant offspring of irradiated parents were frequently sterile. In order to ascertain whether sterility of variant individuals, resulting from treated germ-cells, was due to a mutation producing the visible variation, or to some non-visible effect upon the irradiated cell, a normal brother was tested for fertility at the same time that the aberrant individual was bred. In case sterility was the result of the visible mutation, there would be a much larger percentage of the aberrant than normal flies sterile. If sterility was produced as a general "physiological" effect, the percentage of sterile males would be approximately the same in the two groups.

Experimental Results

Sterility of Treated Individuals

Since the data concerning the sterility of treated individuals is summarized in Table I, only a brief review is required to illuminate any points which remain obscure. Since individual matings were made in every case, the number of sterile cultures in a group represents the number of sterile individuals. Hence, the percentage of sterile individuals may be obtained by dividing the ster-

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TABLE I

STERILITY OF TREATED FLIES

Sex	Age at time of treatment							
	Adults		71–72 hour larvae		35–36 hour larvae		Controls	
	Male	Female	Male	Female	Male	Female	Male	Female
Number	550	487	547	625	968	552	401	351
Fertile cultures	439	388	164	447	253	299	329	305
Sterile cultures	111	89	383	205	715	253	72	46
Per cent. sterile	20	18	70	31.4	73.8	46.4	18	13.1
	±1.2	±1.1	±1.3	±1.2	±.94	±1.43	±1.18	±1.18
Difference in per cent. sterile in experimental and control	2	4.9	52	18.3	55.8	33.3	.....	.....
	±1.7	±1.6	±1.7	±1.7	±1.5	±1.5	.....	.....
Difference divided by P. E.	1.17	3.06	30.6	10.7	37.2	22.2	.....	.....

ile cultures by the total number of cultures tested in that particular group.

In the group of control females, 351 females were mated individually and forty-six of the cultures were sterile. Therefore, 13.1 ± 1.18 per cent. of the control females were sterile. The percentage of sterile individuals found among control males was slightly higher than that of the females. This group contained 401 cultures, seventy-two or 18 ± 1.18 per cent. of which proved to be sterile.

The males and females treated as adults show very little increase in sterility over the controls. From the group of males treated after metamorphosis, 111 of the 550 cultures were sterile. This figure represents 20 ± 1.2 per cent. of the individuals. If we compare the sterility of individuals in this group with that found in control males, the difference appears as 2 ± 1.7 per cent. This difference is 1.17 times its probable error and is undoubtedly without significance. On the other hand a slightly greater difference appears when females treated as adults are compared with the control females. As may be

seen from the table, eighty-nine, or 18 ± 1.1 per cent. of the 487 females, were sterile. This percentage of sterility differs from that found in the control females by 4.9 ± 1.6 per cent., a difference which is 3.06 times the probable error and slightly significant.

The sterility produced by treating larval stages is vastly different from that obtained, either by treating adults, or from untreated controls. Males developing from larvae treated at 71–72 hours of age numbered 547; 383, or 70 ± 1.3 per cent. of these males, were sterile. This percentage differs from the percentage of sterility found in the control males by 52 ± 1.7 per cent. This difference is 30.6 times the probable error and the significance is without question. Females obtained from larvae irradiated at 71–72 hours of age are decidedly more fertile. Among the 625 females of this group 205, or 31.4 ± 1.2 per cent., were sterile. This sterility varies from that of the control females by 18.3 ± 1.7 per cent. This, no doubt, represents a significant variation since the difference is 10.7 times the probable error, but is a much lower sterility than that found in males treated in the same stages of development.

In a consideration of the sterility of flies treated at the larval age of 35–36 hours it is desirable to mention the mortality of pupae. Cultures treated at 35–36 hours of age appear to develop normally through pupation. Pupae form abundantly in such cultures, but only about 25 to 50 per cent. of the pupae produce imagoes. Irradiation of the young embryos produces some effect which inhibits metamorphosis in from 50 to 75 per cent. of the cases. This high rate of mortality seriously affects the ease with which one may obtain a large number of flies for mating.

Larvae treated at 35–36 hours of age supplied 968 males, 715 of which were sterile. This sterility represents 73.8 ± 0.94 per cent. of the P_1 flies of the group. If the 18 ± 1.18 per cent. of sterility found in control males is subtracted from this figure a difference of

55.8 ± 1.5 per cent. is obtained. This difference is highly significant, being 37.2 times its probable error. The sterility found among females treated at 35–36 hours of age is not nearly so high as that of the males. Among the 552 cultures treated at this age 253 failed to reproduce. The percentage of sterility in this case is 46.4 ± 1.43 per cent., and differs from the sterility of control females by 33.3 ± 1.5 per cent., a significant difference equal to 22.2 times its probable error.

STERILITY OF VARIANT F_1 MALES *vs.* STERILITY OF NORMAL BROTHERS

In attempting to determine whether or not the sterility of variant flies derived from treated germ-cells is associated with the phenotypic variation several normal males, one parent of which had been irradiated, were tested. For 427 of the variant individuals tested for mutant characters 427 normal brothers were tested for fertility. Among the 427 normals, fifteen were found to be sterile. These fifteen sterile individuals compared to the total number of normals tested reveal a sterility percentage of 3.5 ± 0.59 per cent. The group of aberrant brothers, equal in total number, contained 105 sterile males. This group exhibited a much greater proportion of sterile flies than the array of sterile individuals found in the group of normal brothers. The percentage of

TABLE II

STERILITY OF F_1 MALES

A Comparison of the Sterility of Variant F_1 Males with that of Normal F_1 Brothers

	Normal F_1	Variant F_1
Number	427	427
Number fertile	412	322
Number sterile	15	105
Per cent. sterile	$3.5 \pm .59$	24.6 ± 1.3
Difference in per cent.	21.1 ± 1.4	
Difference divided by probable error	15.07	

sterility of the latter group is 24.6 per cent. with a probable error of 1.3 per cent. Table II summarizes the data comparing the sterility of variant F_1 flies with that of their normal brothers.

Discussion

The data dealing with the degree of sterility induced by irradiation of *Drosophila* require no consideration beyond pointing out that all treated groups, except males treated as adults, contain sterile flies as a result of x-ray treatment (Table I). The sterility of this exceptional group differs from the sterility of control males by 2 ± 1.7 per cent. The difference is of little consequence, being only 1.17 times the probable error. The percentages of sterile flies found in all other groups are significantly different from that found in the controls. These results are in agreement with the findings published by the writer in 1932, save that due to small numbers considered in the publication, some of the groups were only highly suggestive in the direction substantiated by the present and more voluminous data.

It has been frequently noted that abnormal offspring of treated parents are sterile. Hanson (1928) reported a very large percentage of the aberrant flies which he obtained from treated male parents as being sterile. The problem arises as to whether the sterility of such variant individuals may be ascribed to some reaction linked with the variation, or to the general effects of the irradiations upon the extra-chromatin content of the cells. To be sure, since such individuals fail to breed, it is not definitely known that the aberrant condition is a result of gene mutation, or even of chromosome abnormality. Many such variations have been referred to as the result of "physiological effects," a term including all but explaining nothing. However, we have little evidence upon which to base any conclusions concerning the possibility of an abnormal individual, typical of mutant characteristics, being produced by factors other than

gene mutation, or chromosome abnormality. If all typical variations are of chromatic origin (disregarding the abnormalities obviously resulting from such causes as pressure, etc., during development) the testing of the fertility of a normal brother for each variant male should determine if the sterility of variant individuals is due to a general effect upon the treated cell, or if it is associated with the chromatic disturbance produced by the irradiations. Data bearing upon the problem are recorded in Table II. The sterility of variant males is 21.1 ± 1.4 per cent. greater than that of their normal brothers. This difference, 15.07 times its probable error, is highly significant. The sterility of the variant organism is undoubtedly associated with the underlying cause of the variation, or is a result of the morphological disfiguration. Hence, if our reasoning is correct, the sterility of flies developing from irradiated germ-cells may be attributed, either directly or indirectly, to disturbances of the chromatin material of the treated cells. These so-called disturbances of chromatin may take the form of gene mutations, translocations, deletions, duplications, or inversions. Perhaps many of the sterile individuals which express visible variations carry chromosomal abnormalities which prohibit the germ-cells from entering into the formation of zygotes. In several cases eggs were fertilized and development proceeded to the formation of pupae but metamorphosis failed to occur. Such cases suggest semi-dominant lethals, associated with autosomes, which occasionally permit the appearance of an individual (accounting for the original) but which allowed no offspring of the original male to metamorphose in the observed cases. Such an assumption is not far-fetched since dominant lethals are known in other forms (Stancati, 1932).

CONCLUSIONS

(1) Irradiation of *Drosophila*, with the light dosage employed, produces sterile flies in all the groups treated,

except adult males. The percentage of sterility is higher if larvae are irradiated than if adults are treated, and more sterility is induced by treating male larvae than by irradiating females while in the larval stages.

(2) The percentage of sterility of variant offspring of irradiated parents is higher than that of the normal offspring of the same parents. This is interpreted to mean that sterility is associated with the underlying cause of the morphological variation.

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PRECIPITINS AND PHYLOGENY IN ANIMALS

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I. INTRODUCTION

THE precipitin reaction can be generally used as an indicator of the chemical similarity of proteins.[1] Such being the case this serological test can be applied to biologic problems which require information concerning protein similarities. There are many such problems. Among them is the problem of determining the relationships of species to each other, which is a great one, still far from satisfactorily solved in most cases. The fact that naturalists of recent times have so often forsaken the study of phylogeny is due more to the feeling that such a study is likely to yield little certain progress than to the belief that the problems of phylogeny are unimportant or sufficiently well analyzed. As a matter of fact the systematic work of to-day consists largely in making revisions of previous works dealing with the same groups, but always using essentially the same methods and principles.

What is most needed in phylogeny is a method of analysis independent of morphology, to be used in a complementary manner as a check on morphologic findings. This newer type of analysis of the constitution and relationships of organisms should be as objective as possible and as quantitative as the investigation of such a complex biological problem as phylogeny can hope to be. Such a method of analysis is available in certain serologic tests, especially the precipitin reaction.

Soon after its discovery by Kraus (1897) the precipitin reaction was extensively used in the study of animal relationships by Nuttall. The results of 16,000 precipitin tests were summarized in Nuttall's book, "Blood

[1] See H. G. Wells, "The Chemical Aspects of Immunity," 1929, for a thorough discussion of immunological specificity.

Immunity and Blood Relationship" (1904). Using almost entirely the serum or egg proteins of Vertebrata, Nuttall discovered that the degree of reaction between a precipitating antiserum for the proteins of one species and similar proteins of other species was correlated with the systematic positions of these species. The most intense reactions occurred when antisera were used with the proteins of closely related species. Little or no reaction was obtained when distantly related proteins were tested. The degree of reaction was thus in general proportional to the closeness of relationship of the species tested and confirmed the results of the earlier systematic work, based mainly on morphology. More recently Carl Mez[2] and his students have carried out extensive serological analyses of plant relationships, using in part the precipitin test, and they too have generally confirmed the results of the earlier plant taxonomy.

The precipitin reaction, then, has generally confirmed the results of the best systematic work and therefore can be trusted to yield significant data in phylogenetic investigations. If the best taxonomic work based on morphology measures or indicates relationship, so then does the precipitin test for the results obtained by both run parallel over a wide range of species. The proteins of the plant or animal body and especially the serum proteins of the latter are unusually favorable material upon which to base conclusions regarding phylogeny. This conclusion has been recently affirmed by Landsteiner (1928) who states, "Thus one could roughly construct the zoological tree merely on the basis of precipitin reactions" (p. 596). But must this test be forever limited to the confirming of previous morphological analyses of relationships? Certainly not, even though comparative anatomy has a chronological start of many centuries over comparative serology. There are other possibilities in the applications of precipitin tests. In the first place, the precipitin reaction can be made quantitative and can

[2] See many papers in *Botanisches Archiv.* from 1922 on.

therefore yield *measurements* of the degree of protein similarity in related species. This will result in making the study of relationships more exact, more scientific, a most desirable achievement. In the second place, there are still large groups of organisms whose interrelationships are uncertain, notwithstanding that their anatomy may have been studied more or less intensively for a century or more. This is due of course to the failure to completely overcome some of the important limitations of morphologic taxonomy. The precipitin reaction may then make contributions of importance to (1) quantitative phylogeny and (2) to the analysis of the relationships of species or larger groups of uncertain systematic position.

II. The Precipitin Reaction as a Basis for a Quantitative Phylogeny

A. Ring Tests

The great majority of Nuttall's precipitin tests were made in a relatively crude manner. Each antiserum was tested in turn with the available antigens. The proteins used as antigens might be fluid sera, or egg proteins; more commonly they were extracted from dried filter paper previously dipped in blood. Only a "foam test" was used to determine whether protein was present in such extracts or not. The antigens then were never known to be comparable in their strengths, a very important consideration. Furthermore only a single dilution of each antigen (usually 1:100 to 1:200) was tested, the more accurate dilution method not being employed because of the labor involved. The degree of reaction was described as "great reaction, marked clouding, medium clouding, faint reaction, no reaction." Needless to say, the results could only be taken as a crude guide to the similarities of the antigens tested.

Some significant improvements have been made in the manner of performing ring tests since Nuttall's work. One of these is the method of testing a whole series of

dilutions of each antigen and recording as the titer the highest dilution in which a precipitate forms after a given time. A technical advance of the greatest importance was the utilization of a reliable method for determining the protein concentration of the antigens tested, so that the results should be really comparable when a series of different antigens is tested. It appears that Sauli (1911) was the first to use the Kjeldahl method for such a purpose. Becker (1919) did likewise, but for the most part serological work has continued to be reported in which there has been a careless disregard of the importance of testing antigens of comparable strengths. Another improvement is the utilization of buffered salines as the media in which the reactions take place. The method of buffering of Evans (1922) has proven very useful for all routine tests, even though there is no marked effect of pH on the reaction within ordinary limits.

Of greater importance is the determination of the errors involved in repeated readings of the same tests, using the same reagents. Except for Nuttall's attempt to determine the range of error of his volumetric technique there has been apparently an almost total neglect of this important matter. It should be well known to every biologist, not exempting serologists, that experimental results of any kind must first be judged on the basis of their inherent reliability.

These technical considerations were fully appreciated and the advances in technique were carefully applied to a study of the relationships of some common Mammalia reported by Boyden (1926). The results of the tests were expressed in quantitative terms. The reaction of an antiserum with the antigen used in its production, the homologous reaction, was designated 100 per cent., following Nuttall's suggestion in regard to his quantitative tests. All the tests with the antigens of related species, heterologous tests, were expressed in per cent. values indicative of their strengths relative to the homologous reaction.

The results obtained are summarized in Table I. In general, they confirmed Nuttall's findings and disproved the opposing claims of Friedberger and Meissner (1923),

TABLE I

TESTS AND MEANS

Species	Per cent. values	M
Dog: Horse	2.4; 3.13; 12.5; 1.56	4.9
Dog: Sheep	4.16; 2.4; 3.1; 12.5	5.5
Dog: Pig	4.7; 4.6; 9.4; (0.8)	6.2
Dog: Beef	16.6; 12.5; 8.3; 4.7	10.5
Beef: Horse	16.6; 6.2; 8.3; 6.2; 6.3; 12.5	9.4
Beef: Pig	16.6; 12.5; 8.3; 12.5; 12.5; 16.6	13.2
Beef: Sheep	66; 75; 50; 50; 100; 75	69.3
Sheep: Horse	4.16; 2.1; 3.1; 8.3; 0.78	3.7
Sheep: Pig	8.3; 6.3; 3.1; 12.5; 8.3	7.7
Pig: Horse	9.4; 2.1; 6.2; 8.3; 1.56	5.5

and others, but the real significance of these quantitative measures of relationship has only recently been appreciated (Boyden, 1932). To understand this we must consider in more detail the methods of determining these quantitative measures of relationship.

The per cent. value of the relationship between any two species is determined by the ratio between the heterologous and homologous titers for a particular antiserum. For example, an antibeef serum, tested with beef serum, had a titer of 1:6000 (the highest dilution in which a precipitate occurred after one hour at room temperature). The same antiserum, tested with sheep serum, had a titer of 1:4000. The per cent. value of the relationship between beef and sheep then becomes $\frac{4000}{6000}$ or 66 2/3 per cent. See Fig. 1.

Now there are two ways of determining this per cent. value for the relationship of any two species, provided antisera for both of them are available. Antiserum against species A can be tested with antigen A and also with antigen B, which will give one measure of the interrelationship. But also antiserum against species B can

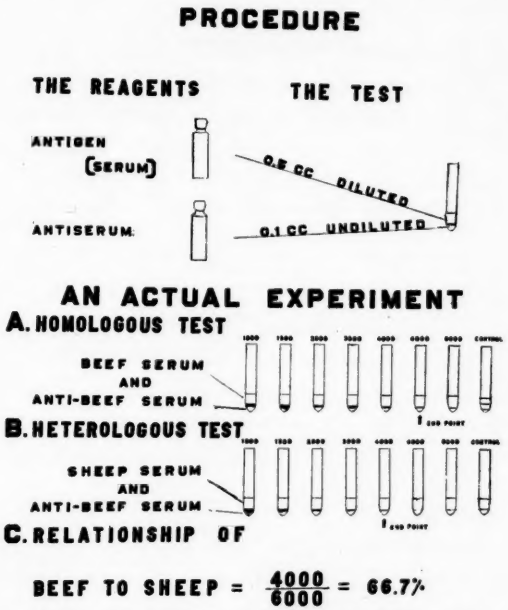

FIG. 1. A chart to illustrate the method of obtaining the per cent. relationship values. The sheep: beef relationship would also be determined in the reciprocal manner, *i.e.*, by testing an antisheep serum against sheep and beef sera.

be tested with antigen B and antigen A, which will give another independent per cent. measure of the relationship between the same two species. In other words, the precipitin tests can be made *reciprocally* and the values so obtained used as a check on each other, for it was discovered that the reciprocal values did generally check, considering the range of error of the individual tests. The principle of reciprocal relationships thus disclosed in precipitin tests may be stated as follows:

The per cent. values of the relationship between any two species A and B should be of the same order of magnitude, whether antiserum A is tested with antigen B or antiserum B is tested with antigen A.

The most evident exception to this principle related to the tests with Rodentia and Lagomorpha. When rabbits

are used for the production of antisera, such antisera react not at all with rabbit blood and only slightly with the blood sera of the related rodents. This inhibition of antibody response occurs whenever an animal is injected with its own or a closely related serum. Consequently, the proper relationship of Rodentia and Lagomorpha to other Mammalia can not be directly determined when rabbit antisera alone are tested. On the other hand, when fowls were used for the production of antisera, there was no such inhibition in the response to rodent antigens. These considerations lead to an important corollary to the principle of reciprocal relationships:

The principle of reciprocal relationships holds only when the antisera are produced by animals which are about equally distant from the species whose antigens are tested.

Bearing these things in mind we may understand the further steps in the utilization of these per cent. relationship values. Since the per cent. values were determined several times and checked reciprocally and since all the values for the relationship between any pair of species are comparable when it is known that the antigens are equivalent in strength, it is possible to average all these values for each pair of species and then to determine the reliability of the mean by the usual statistical methods. Such mean per cent. values (M) for the species tested reciprocally are given in Table II, together with their probable errors. Since the least reliable value (dog *vs.* horse) is still 3.3 times its P.E., it is probable that the whole series of values is statistically significant. We have, then, a series of *measurements* of the mean relationship values of these species, of known reliability. We might expect such measurements to have some unusual biologic significance. That such is the case becomes apparent when these values are used to graphically express the interrelationship of these Mammalia.

It is customary and natural in phylogenetic trees to put closely related forms near together and more dis-

TABLE II

AVERAGE RECIPROCAL VALUES OF MAMMALIAN SERA (M) TOGETHER WITH THEIR PROBABLE ERRORS, AND THE VALUES OF 100–M FOR ALL THE SPECIES TESTED RECIPROCALLY

Species	M (per cent.)	P.E.$_M$	100–M
Dog *vs.* horse	4.9	± 1.49	95.1
" " sheep	5.5	± 1.37	94.5
" " pig	6.2	± 0.87	93.8
" " beef	10.5	± 1.5	89.5
Beef " horse	9.4	± 1.08	90.6
" " pig	13.2	± 0.78	86.8
" " sheep	69.3	± 4.7	30.7
Sheep " horse	3.7	± 0.79	96.3
" " pig	7.7	± 0.92	92.3
Pig " horse	5.5	± 0.98	94.5

tantly related forms proportionally farther apart. In these quantitative precipitin tests, however, the higher values indicate greater protein similarities and genetic relationships. Since 100 per cent. indicates practical identity, the values of 100-M are to be chosen as the linear distances between the species when expressed in the form of a phylogenetic tree.[3] But when the actual preparation of such a tree was undertaken, it was found that the values would not fit with each other on a plane surface (unless broken into segments)! This was a most disturbing result. Some time later, however, it was discovered that those same values would fit very well in three dimensions! So, finally, a wooden model was constructed, to show in a spatial arrangement the interrelationships of these five species of Mammalia. Fig. 2 shows this model. Its dimensions were determined from the 100-M values of Table II, and in Table III are given the calculated "tree" values ($\frac{100-M}{2}$) in centimeters, together with the actual dimensions of the model itself and the per cent. of difference between the calculated and

[3] Actually the values $\frac{100-M}{2}$ in cm were taken in order to make a model of convenient size.

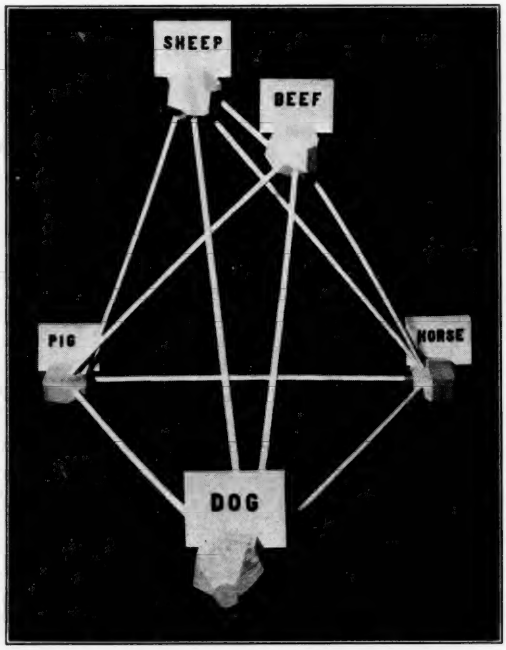

FIG. 2. Model showing in three dimensions the interrelationships of 5 species of Mammalia based on their $\frac{100-M}{2}$ values. This is a graphical expression of present relationships. It is no simple guide to ancestry.

actual values. The model agrees with the calculated values with a maximum error for the distance between any two species of 9.9 per cent. and an average error for all the distances of 6.2 per cent. The agreement, then, is all that could be expected. It should be noted also that the locus of each of the five species is determined by four distinct relationship values and that therefore their agreement must be of some unusual biologic significance.

The quantitative precipitin tests can then give data of relationships which check reciprocally with themselves to a surprising degree. Such data have forced a three-dimensional expression for the present relationships of the Mammalia tested. In other words, this tree of relationships has grown in three dimensions like any

other tree. It is therefore not only a more accurate but, withal, a more natural manner of expressing relationship data. How broadly these principles are applicable to other species remains to be discovered. A study of amphibian relationships by the writer in cooperation with Dr. G. K. Noble (1933) has yielded a three-dimensional figure for the relationships of four species of Caudata. Here the data again forced a three-dimensional expression. Now it may well be that further researches will show that even a three-dimensional figure can not adequately express the complex interrelationships of large groups of species. If so, other methods

TABLE III

CALCULATED AND ACTUAL "TREE" VALUES

Species	Calculated tree $\frac{100-M}{2}$	Actual tree CM	Per cent. diff.
Dog: Horse	47.5	51.0	7.4
Dog: Sheep	47.3	52.0	9.9
Dog: Pig	46.9	51.0	8.7
Dog: Beef	44.7	44.5	0.5
Beef: Horse	45.3	41.5	8.4
Beef: Pig	43.4	47.5	9.4
Beef: Sheep	15.3	15.0	2.0
Sheep: Horse	48.1	51.5	7.1
Sheep: Pig	46.2	46.0	0.4
Pig: Horse	47.2	51.0	8.1
		Av.	6.2

of expression will need to be devised. Whatever the method of graphical expression of these data, it should be emphasized that the data themselves are the most significant results of these studies. Statistically reliable measurements of protein similarity and hence of relationships, these are the facts of prime importance.

Knowledge of phylogeny is of two chief kinds: (1) that regarding the present relationships of existing species; (2) that regarding their ancestry. To construct a complete phylogenetic tree requires both kinds of knowledge. Heretofore quantitative measures of present relationships have been almost entirely lacking, and information

as to ancestry has been even less certain. It begins to appear, however, that precipitin tests can give us quantitative measures of the present relationships of existing forms and thus supply exact ideas as to that part of their phylogeny which concerns their present position in the phylogenetic tree. If so, the tests will have succeeded in giving us what a century or more of intensive morphological investigations has failed to provide, namely, a basis for a quantitative phylogeny.

When three dimensions[4] (precipitin data may eventually require even more) are needed to express present relationships, the graphical expression of ancestry becomes more difficult. If we may consider time as the fourth dimension (all evolution occurs in time), we might show ancestry along a fourth or time axis. It sometimes happens, however, that living organisms possess traits characteristic of ancient times. These traits may as likely be serological as morphological. The fact is that the serological characteristics of higher organisms are in general conservative. These organisms, then, though existing in the present, may represent ancestral qualities, and such species may serve as the basal loci in such phylogenetic polyhedrons. In the paper by Boyden and Noble above referred to, *Cryptobranchus* serves as the basic form, representing ancestral qualities. It is not meant that the other Caudata have descended directly from the *Cryptobranchus* we know to-day along the lines connecting it with the other species. What is shown in such a model is a graphical expression of the present interrelationships of these species to an acknowledged primitive species and to each other. With this more exact expression, the actual course of descent may eventually be more certainly determined.

[4] Carl Mez has suggested (correspondence) that these values may be expressed in the usual way if the per cent. values are broken into segments which lie along the trunk or as branches of the phylogenetic tree.

B. Volumetric Quantitative Tests

Nuttall published in his book, in addition to the qualitative tests, the results of 500 quantitative tests in which the volume of precipitate was measured. Nuttall and Strangeways claimed a 10 per cent. error for these tests, but actually the error was a much larger one when different antigens were used because of the failure to determine their protein concentrations and make them equivalent. In fact, the errors seem to be of the same order of magnitude as those involved in the ring technique used by Boyden.

The method of determination of the volume of precipitate was slow and laborious, inasmuch as the precipitate was allowed to settle of itself until a constant volume was reached. A more rapid volumetric technique utilizing the centrifuge to throw down the precipitate has been described by Schur (1904), by Hamburger (1905), by Mollison (1924) and by Boyden and Baier (1929). Of these workers only the latter have given an adequate statement regarding the inherent reliability of the measurement of volumes of precipitate. In one series, the volumes of precipitate formed by the interaction of a definite amount of antiserum and antigen was determined 36 times. It was found that the average error of the individual readings was 5 per cent. when compared with the mean of the whole series. The average deviation of the means of successive pairs from the mean of the whole group was the same, while the average deviation of the means of the values taken in quartets dropped to 3 per cent. It is definitely known, then, that the volume of precipitate can be determined with as great a reliability as could reasonably be expected for such a complex colloidal reaction.

The results of the application of such a technique to the study of serologic relationships should be of great interest. It is likely that this technique will succeed in distinguishing closely related species, which have heretofore been indistinguishable by the precipitin test. Un-

fortunately, little has as yet been done with its aid. Some indication of the type of result attainable is to be had from the results of experiments shown in Figs. 3 and 4.

Fig. 3 shows the curve obtained when an antipig serum

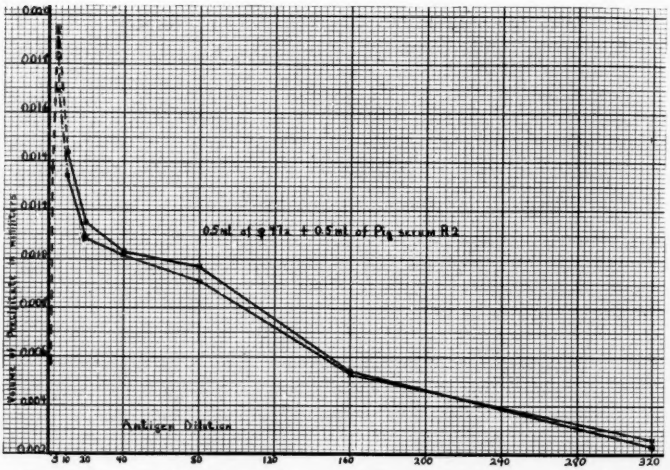

FIG. 3. The results of the volumetric measurement of precipitate in a repeated titration of an antipig serum with pig serum. The region of maximum precipitate is shown in broken lines because the errors of reading are usually greater in this part of the range of precipitation. An interval of two weeks intervened between the first titration (circles) and the second.

(♀ 47a) was titrated against increasing dilution of the homologous antigen (pig serum R2). The whole series of readings was repeated after two weeks and the agreement of these two curves is very good, as expected. The region of maximum precipitation and the prozone to the left of it are subject to the greatest errors and are represented therefore in broken lines. The right wing of the curve shows a decrease in volume of precipitate with increasing dilution of antigen, which however is not perfectly regular. The curve is a more complete and exact expression of the results of the interaction of an antiserum and the homologous antigen than the ring test can

give which limits itself to the end point only. Really the whole range of antigen dilutions within which precipitation occurs should then be used in defining the precipitating powers of an antiserum, whether the tests are homologous or heterologous.

Now when an antiserum is tested in this way with both homologous and heterologous antigens the result was a double curve, as shown in Fig. 4. Here an antibeef

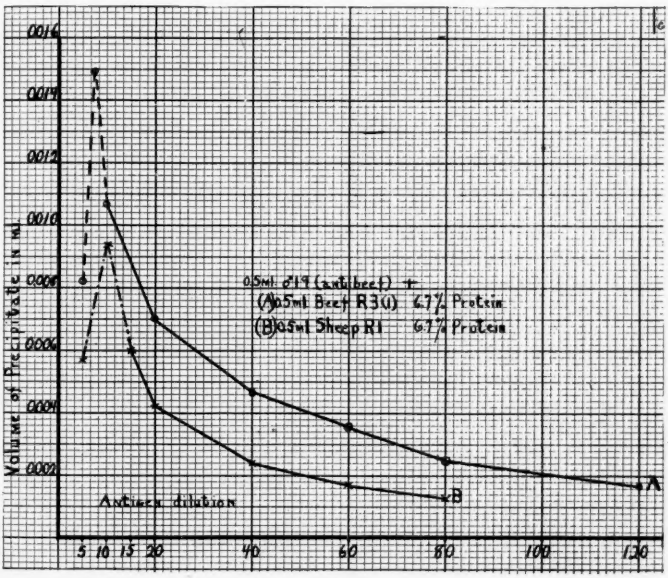

FIG. 4. The volumes of precipitate resulting from the interaction of an antibeef serum with beef serum (A) and with sheep serum (B) of the same strength.

serum (♂ 19) was tested against a beef serum ($R3^1$) and then against a sheep serum (R1) of the same protein concentration. Curve A representing the homologous test is similar to curve B but almost uniformly greater throughout its length. Here is a different type of graphical expression of the degree of protein similarity and hence of genetic relationship of these two species. By

comparing the areas under corresponding parts of these two curves one might get a much more accurate expression of the relationship value than is possible by means of the ring test. These results are, however, suggestive only. Would the reciprocal volumetric test yield the same curves but in reversed position? If so the principle of reciprocal relationships would be verified in a most exact manner. What types of curves will result from studies of different groups of organisms? These and other important questions must await further progress in this field of research. The recent work of Baier (1933) has provided a solid foundation for further developments.

III. The Precipitin Reaction as a Guide to the Relationships of Species whose Systematic Positions are Still Uncertain

According to Noble (1931) there are still some doubts as to the systematic positions of certain Amphibia. In a recent paper (Boyden and Noble, 1933) it is pointed out that Siren and Necturus are two such forms. The morphological and life history evidence as to Siren is conflicting, and it was impossible to determine the nearest relatives of the sirenids by it. Necturus was somewhat less of a puzzle, but here again the available data were not capable of giving a complete answer as to its precise systematic position. The precipitin tests, however, disclosed that Siren was more distant from the primitive Cryptobranchus than either Amphiuma or Necturus, and that Siren, Amphiuma and Necturus all stood fairly close together. The precipitin tests then indicated that Siren and Necturus were related to Amphiuma and to Triturus and that they had probably evolved from a salamandrid stock, rather than from a hynobiid stock. This appears to be a satisfactory solution to a phylogenetic problem of long standing.

Results of possible value were obtained also with the Ranidae. The precipitin tests indicated that *Rana*

catesbeiana and *R. pipiens* were not so closely related as one would assume because of their inclusion in the same genus. *Rana clamitans* stood very close to *R. catesbeiana*. However, there seems to be little morphological data to justify the generic separation of catesbeiana and pipiens, although Hadley (1929) has found a marked incompatibility in grafts between the skins of clamitans and pipiens, and thus indirectly supports the serological findings. Further tests are necessary, however, before one may proceed to outline the subgroups within the genus Rana, and the precipitin tests so far reported are to be considered as merely suggestive of the possible results of further experiments.

No doubt the greatest contribution which may be expected from the use of the precipitin test in the study of animal relationships will come when the heterogeneous assemblage of Invertebrata is consistently attacked. It is a telling exposition of the limitations of morphological taxonomy to realize that we still have whole phyla of "uncertain systematic position" among these Invertebrata. Here is a field for serological investigation which must soon be carefully tilled. An indication of the value of the results attainable in this field is the observation of Nuttall that Limulus is more closely related to Arachnida than Crustacea, though at first it was considered more closely related to the latter. Hektoen (1926) has obtained a few cross reactions with helminth parasite extracts. A brief note by Brown and Heffron (1928) describes the results of a few tests with butterfly antigens. Canning (1929) has studied the precipitin reactions of various tissues of *Ascaris lumbricoides* and related helminths. Erhardt (1931) has summarized the researches in the whole field of the serological study of animal relationships, and reported his own investigations. His paper will be very useful for all workers in this field. These results are, however, no more than the beginning of a serological attack on problems of invertebrate relationships. Further work here will probably

help to obtain the solutions of many phylogenetic problems of considerable importance.

IV. The Relative Values of Morphologic and Serologic Data Concerning Relationships

Though it is much too early to attempt anything like a final appraisal of the relative value of the data produced by these two independent methods of investigation, there has been work enough done to justify a first estimate.

The great bulk of data used in the study of relationships is morphological. Such extended use of the morphologic technique in taxonomy has disclosed many serious limitations. The fundamental assumption made by all workers in this field is that similarity in structure is in general a guide to relationships and to ancestry. Unfortunately, the working out of this principle becomes exceedingly complicated when actually practised. For there are difficulties due to the existence of non-hereditary structural similarities, to the occurrence of dimorphism and polymorphism, to the existence of complicated life cycles, to the fact of convergence, to the subjective nature of the estimate of similarity, to the very complexity and variety of the structures themselves and the fact that different organs and organ systems may not be equally conservative, and to the general inapplicability of quantitative methods. With all these limitations, it is indeed remarkable that there is as much general agreement among naturalists as actually does exist regarding taxonomy.

The precipitin technique has not been thoroughly tested as yet and its limitations are doubtless greater than they seem at present. To date it is realized that the most serious limitation of all may be the necessary restriction of the tests to existing species. This cuts off the whole realm of extinct life except for the very unusual occasion when an animal or plant may be preserved in ice. In these cases the forms are relatively recent. Besides, the precipitin technique is laborious when care-

fully performed and most unlikely to be a part of the training of those most interested in taxonomic work. The existence of fluctuating variability in the proteins of individuals has not been adequately investigated. On the other hand, the precipitin technique can give fairly consistent results. Table IV gives a summary of the

TABLE IV

RECIPROCAL VALUES OF MAMMALIAN SERA (PER CENT.) COMPARISON OF PRIMARY DATA

	Nuttall	Boyden	Wolfe
Beef *vs.* sheep	56	69.3	40.6
" " pig		13.2	26.6
" " horse		9.4	12.5
Sheep " pig	10.3	7.7	28.1
" " horse		4.4	10.2
Pig " "	17.0	5.5	3.9

average values of the corresponding reciprocal tests obtained by Nuttall, by Wolfe (1929) and by Boyden. It may be seen that the quantitative values are of the same order of magnitude throughout. On the other hand, Wolfe (1933) finds a relatively poorer check of the reciprocal values involving carnivores, a finding which agrees with the lesser reliability of the mean reciprocals in my tests with dog sera. This requires further analysis. Dr. M. F. Guyer has suggested that these discrepancies in Carnivora may be related to the diet, for it is known that the proteins consumed may persist in part unaltered for some time in the body of the host.

Again it has been claimed by certain workers that occasionally an antiserum will react more powerfully with some other protein than the homologous one. But such an occurrence is relatively rare. Nor are there commonly irregularities in the test, though the class of antigens described by Landsteiner as haptens may be widely and somewhat irregularly distributed in nature. An occasional aberrant serological test need no more destroy our general confidence in it as a technique of

taxonomic investigation than the sudden appearance of a monster need cause an abandonment of the principles of morphologic taxonomy.

Each of these methods of taxonomic analysis has therefore its advantages and disadvantages and the two methods should rightly be thought of as complementary. Morphology is very conservative among the lowest organisms, bacteria, but here serological methods are exceedingly delicate and capable of indicating very fine serological races. At the other end of the living world morphology becomes very special and serology very conservative. No better combination of advantages for the whole realm of phylogenetic investigation could be thought of. Morphology may err in failing to detect convergence; here it is very probable that serology can help to indicate the true relationships of the species studied. Morphology may confuse a primitive condition with one specialized in the direction of degeneration; serology will likely be able to aid here also. Morphology can marshall its fossils; serology can lead to a more exact and quantitative estimation of present relationships. These facts all indicate that the serologic method of investigation may eventually come to be considered as important in phylogeny as the morphologic method, but before that time will come there must be a great deal of careful, quantitative, serologic research. To those who are willing to learn to master this newer method of attack on the old problems of relationships, the outlook to the future seems bright.

CONCLUSIONS

(1) The need for an independent method of phylogenetic analysis may be supplied by serology.

(2) The precipitin test has yielded quantitative measures of the present relationships of certain Mammalia and of some common Amphibia.

(3) These relationship values have been shown to be

statistically significant and to require expression in three dimensions.

(4) The serologic and morphologic data complement each other and may eventually come to be considered of about equal value in phylogenetic research.

(5) The extension of the serologic attack to the Invertebrata should yield results of great significance.

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EVOLUTIONARY RELATIVE GROWTH IN THE TITANOTHERES

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IT has been shown (See Huxley, 1932) that the relation of a part to the whole, or of a part to another part of the organism, as the organism increases in size, conforms to the power function, $y = bx^k$. This relative growth function has been applied almost entirely to ontogenetic data. It is applicable also to the average values of phenotypic quantities of genetically diverse but related groups. In this case a point on the graph represents the group rather than the individual, although to be sure the group may be typified by a single specimen. The group is the unit rather than the individual. In this way the relative growth function has been found to apply to data of extant and extinct groups of animals, as reported in several preliminary abstracts (Hersh, 1931b, 1932; and Robb, 1932).

When the function is applied in the way indicated to group data, the constants b and k become genetic constants distinctive for the characters of the groups for which they have been determined. In regard to extinct groups, where there is an orthogenetic trend, b and k are also constants of phylogenetic relative growth. This paper concerns mainly the application of the relative growth function to various skull measurements of the titanotheres (Osborn, 1929). It is found that the evolutionary increase in size, on a basis of a comparison of the adults of different species, is partitioned to different organs in accordance with the same function. k in such cases is a ratio of relative rates of evolutionary increase and may be called a coefficient of phylogenetic growth-partition.

The zygomatic width/basilar length relation. When the zygomatic width (y) is plotted against the basilar

length (x) of the skull for all the specimens (some 117) for which data are given in Chapters V and VI of Osborn's monograph, the points fall within a broad band whose edges are approximately parallel, and which is

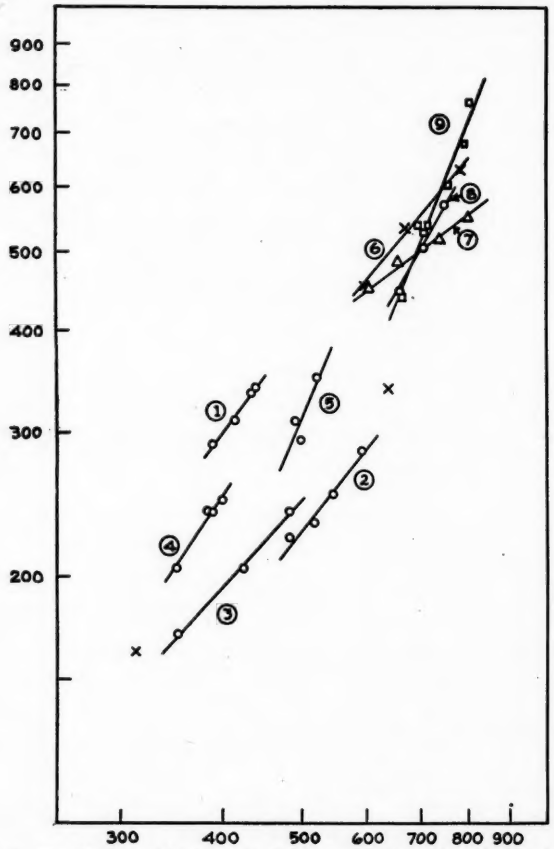

FIG. 1. Double logarithmic plot of the zygomatic width (y) against the basilar length of the skull (x), showing fitted lines for certain of the better known genera. Eocene genera as follows: 1, Palaeosyops; 2, Dolichorhinus; 3, Mesatirhinus; 4, Metarhinus; 5, *M. manteoceras*. Oligocene genera: 6, Brontops; 7, Menodus; 8, Allops; 9, Brontotherium. Each point on the fitted line designates the value for a single species. The cross at 313 (x), 162 (y) is for *Eotitanops borealis;* the one at 640 (x), 340 (y) is for the aberrant *Allops walcotti.*

further characterized by a break into two segments, one of which represents the Eocene and the other the Oligocene titanotheres. The break occurs in a region which represents 200 feet of unexplored deposits. While the width of the break is no doubt accentuated by this fact, yet there is reason for doubting whether the break itself is merely an expression of the gap in the data. It may be seen from Fig. 1 that the lines representing various genera of titanotheres show a change in slope at the break in the ribbon. Furthermore, data (Wagner, 1930) on the same characters of the skull of some 35 races of dogs show a somewhat similar break.

In Fig. 1 the points for genera with less than three species are not included and for those species which included a number of specimens the average value for the characters is the only point indicated. As pointed out above the group is taken as the unit. Each line in the figure represents a single genus for which the data are adequate, in the limited sense indicated, for determining the values of the constants of the function. These values of b and k for various genera are given below in Table 1.

If we accept the view-point that evolution has proceeded by the selection of mutations, then it may be said that the mutations which became incorporated into the genus with the passage of time, and which the paleontologist recognizes as species, are mutations for increase in size without change in b and k. Other accumulated mutations, which by their incorporation into the family brought about divergence into genera, are such as cause a shift in both b and k. So these constants of the relative growth function may be considered genetic constants descriptive of the various genera in regard to the characters under consideration. The results may be summed up in the following tentative rules: (1) Within the genus b and k are constant. (2) Genera are distinguished from one another by differences in b and in k.

If such relationship should prove to be rather general in the study of group variability, the relative growth

TABLE 1

VALUES OF b AND k FOR THE TWO SETS OF CHARACTERS FOR WHICH THEY HAVE BEEN DETERMINED FOR VARIOUS EOCENE AND OLIGOCENE GENERA OF TITANOTHERES, ARRANGED ACCORDING TO INCREASING VALUES OF k. AS k INCREASES ARITHMETICALLY b DECREASES GEOMETRICALLY.

Zygomatic width (y)—basilar length of skull (x)

Genus	k	b
Menodus	0.751	3.659
Mesatirhinus	1.106	0.256
Brontops	1.201	0.213
Dolichorhinus	1.254	0.093
Palaeosyops	1.348	0.092
Metarhinus	1.483	0.035
Allops	1.907	0.0019
M. manteoceras	2.414	0.00009
Brontotherium	2.535	0.00003

Upper molar series (y)—premolar-molar series (x)

Genus	k	b
Megacerops	0.821	1.828
Dolichorhinus	0.903	1.006
Allops	0.959	0.773
Menodus	0.974	0.732
Manteoceras	0.981	0.640
Limnohyops	0.986	0.638
Mesatirhinus	1.071	0.394
Brontotherium	1.079	0.401
Brontops	1.092	0.365
Metarhinus	1.199	0.219
Palaeosyops	1.280	0.137

function is obviously of importance to the systematist in his determination of the relationship between groups. In regard to the characters under discussion these rules undoubtedly hold and so may be used to suggest, in so far as these characters enter into the determination, the systematic position of a somewhat aberrant specimen. The cross in Fig. 1 at 640 (x), 340 (y) represents a single specimen (not included in the calculations) at present referred to the genus Allops under the specific name *walcotti*. In regard to this specimen Osborn says (p. 509),

"While its reference to Allops requires confirmation, its nearer affinities appear to be with this genus rather than with Brontops or Menodus."

It can be seen in Fig. 1 that this specimen departs rather widely from line 8 for the genus Allops, and that it departs still further from the lines for Menodus and Brontops. The specimen most nearly approaches line 9 which designates the genus Brontotherium. Hence the application of the relative growth function to these characters suggests that the specimen is a Brontotherium. It should be borne in mind, however, that all specimens for any genus fall in a band of greater or smaller amplitude on each side of the lines shown in Fig. 1. These remarks on *Allops walcotti* are intended merely as an illustration of the use to which the relative growth function may be put in taxonomic studies, and in the light of rules (1) and (2). It is of further interest in regard to this specimen that Marsh originally classified it as a Brontotherium (Osborn, p. 510).

For a comparison of different species in regard to the characters under consideration, the usual method has been to compare them by the use of the cephalic index. This index is the ratio that the zygomatic width of the skull bears to the basilar length. Skulls are classified as dolichocephalic when the ratio is under 60, brachycephalic when its value is over 70, and mesaticephalic when its value is between 60 and 70. The cephalic index for a particular species may fall below 60, and species of the same genus but later in time may likewise be below 60, and consequently such a genus is dolichocephalic. In terms of the relative growth function, when $k < 1$, the basilar length of the skull is increasing more rapidly than the zygomatic width. The cephalic index is thus progressively decreasing although its value for any one or all species may be either dolichocephalic or brachycephalic. When $k > 1$ the skull is progressively brachycephalic. If the earlier species of the genus are brachycephalic, then the later species are more

markedly brachycephalic. If the smallest species is dolichocephalic, the genus progresses toward the brachycephalic condition. In Brontotherium, for example, the earlier species are dolichocephalic, later species are mesaticephalic and toward the end of the lower Oligocene times at the time of extinction, this genus was extremely brachycephalic. One specimen of *B. platyceras* shows a cephalic index of 110. When $k < 1$, the skull is progressively dolichocephalic. The only genus falling in this class among those for which the value has been determined is Menodus, for which k equals 0.75.

In a discussion on the evolution in length and width of the skull in these animals Osborn says (p. 784), "In Brontops the skull gains 31 per cent. in length and 39 per cent. in breadth. . . ." These numerical values are obtained by a comparison of the type specimens of the earliest and latest species in the genus. Consequently in Brontops, by this use of the type specimens of the smallest and largest species, the zygomatic width increased 8 per cent. more than the basilar length. In terms of relative increases, the width increased 1.26 times, or 126 per cent., faster than the length.

The comparison made in the quotation is tantamount to a use of the relative growth function. One of the ways in which the relative growth function can be stated, and which we may use here since it fits the quotation better than some other way, is as follows: If a variable x increases by a certain fixed per cent., another variable y increases likewise by a certain fixed per cent. As the per cent. increase in x is taken smaller and smaller, the ratio of the per cent. increase in y to the per cent. increase in x approaches a limit, which is the value of k.

For Brontops, the genus mentioned in the above quotation, the value of k calculated from the data using all the male specimens is 1.20. The difference between 1.20 and 1.26 is due mainly to the fact that the former is the value of k in the limit and the latter value corresponds rather to the slope of a line drawn as a secant through a

point of a curve instead of the slope of the line drawn tangent to the curve at the same point. Similar comparisons to that made in the above quotation give values which vary from that given by k, sometimes for the reason stated and sometimes no doubt largely as a matter of random sampling. This would seem especially to be the case in regard to Menodus (Osborn, p. 784).

For some purposes it is better to compare species on the basis of the relative growth function rather than in terms of the various indices. This comparison can be made in terms of the first derivative of the function

$$\frac{dy}{dx} = k\frac{y}{x}.$$

For the characters under discussion it states the increase in the zygomatic width of the skull in mm for an increase of 1 mm in the basilar length. In Table 2 are given the values of the first derivative for ascending species in the four great Oligocene genera. For Brontops, Allops and

TABLE 2

FIRST DERIVATIVES OF THE RELATIVE GROWTH FUNCTION FOR ASCENDING SPECIES IN THE FOUR GREAT OLIGOCENE GENERA OF TITANOTHERES. x, BASILAR LENGTH OF SKULL IN MM; y, ZYGOMATIC WIDTH IN MM; $\frac{dy}{dx}$, THE INCREASE IN WIDTH PER MM INCREASE IN LENGTH

	x	y	$\frac{dy}{dx}$
Brontops brachycephalus	598	454	0.912
dispar	670	534	0.957
robustus	782	631	0.969
Allops marshi	660	447	1.292
serotinus	707	505	1.363
crassicornis	750	570	1.449
Menodus heloceras	603	450	0.560
proutii	655	485	0.556
trigonoceras	739	518	0.526
giganteus	801	550	0.516
Brontotherium leidyi	665	440	1.677
hypoceras	715	540	1.915
gigas	793	679	2.171
platyceras	804	763	2.406

Brontotherium, it may be seen that with increasing size in the genus the width progressively increases, although in Brontops the increase in width may be less than in length. In the case of Menodus as the course of evolution progressed through the species on the way to further increase in size, the incorporated mutations which brought about an increase of 1 mm in the length of the skull brought about an accompanying smaller and smaller increase in the width. That is, the increase in width was relatively less in the larger species of later Oligocene time than in the smaller species of earlier Oligocene time. Menodus is the only progressively dolichocephalic genus.

The upper premolar-molar series/basilar length relation. In Fig. 2 are plotted the values of the total length of the upper premolar-molar series of teeth (y) plotted against the basilar length of the skull (x), in conformity with the relative growth function. The points fall in a broad band which may be interpreted as having the same slope for the Eocene as for the Oligocene titanotheres. The slope of the entire band as indicated by the boundary lines which are drawn in the figure is about 1.4, so that for the titanotheres taken as a single group, the length of the upper premolar-molar series of teeth increased about 1.4 times faster than the basilar length of the skull. The points for the different species were so scattered that it seemed inadvisable to compute the values of the constants in the relative growth function for the different genera. Plotting by genera indicates, however, that the different genera occupy this band in such a way as to give different values of b and k.

The upper molar/total premolar-molar relation. A graph very similar to Fig. 2 is obtained by plotting the length of the upper molar series (y) of teeth against the total length of the premolar-molar series (x). The slope of the entire band in this case is about 1.0. For these characters there are enough specimens in the different species to perhaps warrant the determination of the

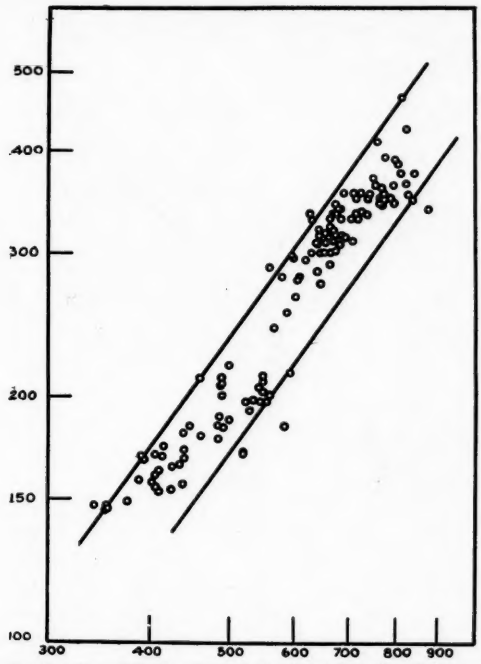

FIG. 2. Double logarithmic plot of the total length of the upper grinding series of teeth (y) against the basilar length of the skull (x). Each point represents the value for a single specimen.

values of the constants b and k in the relative growth function to which the data obviously conform. Fig. 3 shows the fitted lines for those genera for which the data were considered sufficient. The value of k ranges from 0.821 for Megacerops to 1.28 for Palaeosyops (see table 1). For those genera for which the value of k is less than unity, the evolutionary increase in the total length of the upper series of grinding teeth has been partitioned in such manner that the premolars participate in the increase to a greater extent than the molars, and for those genera in which k is greater than unity, the molars participate to a greater extent than the premolars in the evolutionary increase in the upper series of grinding teeth. The relative extent of the greater or smaller participation in the

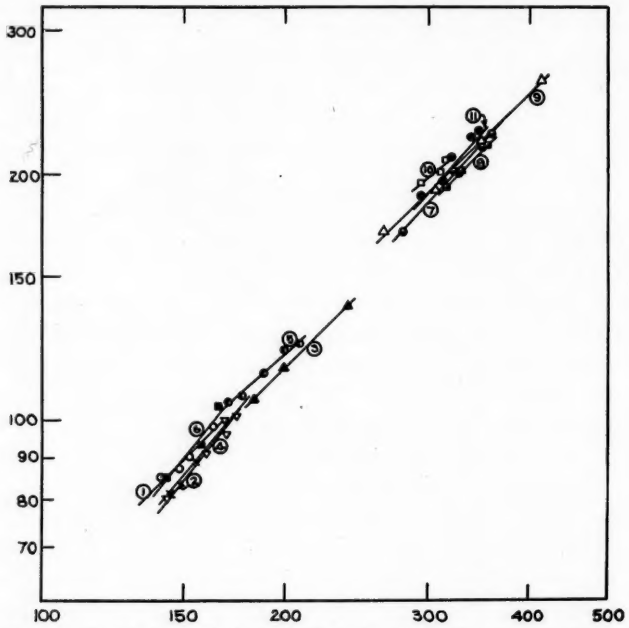

FIG. 3. Double logarithmic plot of the length of the upper molar series (y) against the total length of the whole premolar-series (x). Each line is for a single genus, as follows: 1, Limnohyops; 2, Palaeosyops; 3, Manteoceras; 4, Mesatirhinus; 5, Dolichorhinus; 6, Metarhinus; 7, Brontops; 8, Allops; 9, Menodus; 10, Megacerops; 11, Brontotherium.

evolutionary increase is given by the extent of the departure from 1.0 in the value of k.

The horn length/basilar length relation. The Eocene titanotheres have either no horns at all or quite small horns in comparison to the Oligocene titanotheres which had horns that increased progressively in length until at the time of extinction of the group they were about fifty per cent. as long as the skull, and so were a quite striking feature of the skeleton. When the horn length (y) is plotted against the basilar length of the skull (x) logarithmically, as in Fig. 4, the points for the Oligocene specimens fall within a broad band which conforms to the relative growth function.

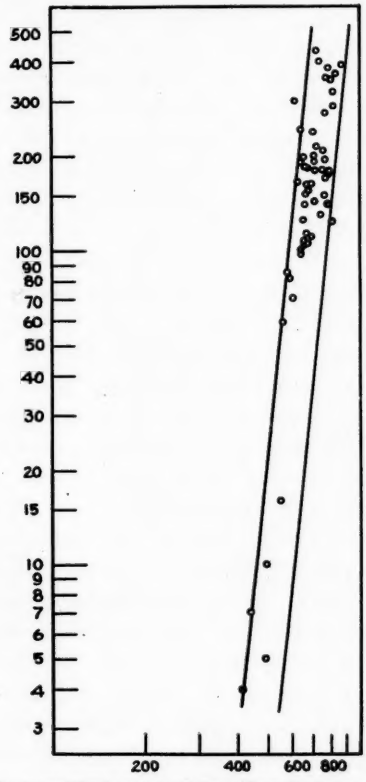

FIG. 4. Double logarithmic plot of horn length (y) against the basilar length of the skull (x). Each point designates a single specimen. The coefficient of phylogenetic growth-partition (k) is about 9.

From the text and the drawings in Osborn's monograph the horn length for several specimens of Eocene titanotheres was estimated. These several points have been included in Fig. 4 where it is seen that they fall within the downward prolongation of the band which was drawn on the basis of the distribution of the points representing the Oligocene specimens.

Consequently, as in the characters discussed previously, for the titanotheres taken as a single group the evolutionary increase in the length of the horns was at

a constant ratio relative to skull length. The value of k for the band is about 9, so that the evolutionary increase in the length of the horns is about nine times the rate of increase in the length of the skull. The points for the different Oligocene genera occupy different positions in the band, but it seems inadvisable to estimate the values of b and k for each genus separately. When the band is extended downward to include the skull length of an early primitive titanothere (Eotitanops, 313 mm), it is found that such a skull should have on this basis a horn about 0.5 mm long.

It seems justifiable to conclude that the germinal organization of the titanotheres at all times possessed the basis for the development of horns. During the course of evolution, selection was in favor of mutations making for larger animals. As the animals increased in size, an approximately constant proportional part of the increase was partitioned to the fronto-nasal horn and this happened to be a quite high figure relative to the increase in the size of the skull, as indicated by the steep slope of the band in Fig. 4. From this point of view then, since the basis for the development of horns was present in all titanotheres, and came to expression with the increase in size of the animals with the incorporation of genes for larger size in any line, it is readily understandable that any titanothere of sufficient size would possess horns. All Oligocene titanotheres had reached this size and so possessed horns.

If we suppose that the value of k for the relation between horn-length and skull-length, instead of being so unusually high, were more nearly a common value in the neighborhood of 1.00, then a titanothere with a skull-length of 800 mm would possess a horn about 1.5 mm long. Even if k should have the quite high value of 2.75, such a specimen would have a horn not longer than about 5 mm, which, considering the broad oval base of the titanothere horn, would most likely fail to be detected as more than a change of contour in the fronto-nasal region of the skull.

Such considerations lead definitely to the conclusions that the mammalian organization may have potentially a mechanism for horn growth, but in many groups the horn does not actually develop because of the particular character of the mechanism, as shown in *b* and *k*, or perhaps merely the size of the organism is such that the horn is off the time axis of development (see Huxley, p. 239).

At any rate the situation in the titanotheres seems clear enough. The titanotheres of early Eocene times did not have horns because they were not large enough. As mutations for larger size occurred and were selected and incorporated into the germ plasm, the successive generations increased in size, and the ontogenetic mechanism for the production of horns was able to produce first incipient horns, and with the passage of generations horns of progressively increasing size were produced. After a certain manner of speaking, these genes which brought about the development of horns may be spoken of as "genes for horns," but certainly not in the sense of representative particles, and not even in the sense of factors of merely local size increase. These genes for horns were factors which brought about an increase in general size, which affected in greater or less degree perhaps all parts of the body. But if we make the assumption that the coefficient of growth-partition for horns had a higher value than similar coefficients for other organs, then it follows that these genes brought about a greater relative increase in the horns than elsewhere in the organism, and so in this restricted sense may be spoken of as "genes for horns." But as suggested above these genes for horns would not have produced noticeable horns if the coefficient of phylogenetic growth partition had had a low value in the neighborhood of unity. The mechanism would, however, have been present.

On this view, then, the germinal organization of these animals was such that the selection of genes for larger size brought about at first very small horns, and as evolution proceeded by further increase in size the horns be-

came progressively larger and larger. As Huxley (p. 219) has urged for such cases, the horns in their incipiency were not directly adaptive. But once the horns had reached a sufficient size to be used as organs of offense and defense, we might reasonably conclude that the animals with larger horns, as a consequence of the presence of horns, were selectively favored at the expense of the smaller-horned animals. Once the horns had reached this size, they would be directly adaptive and so have survival value. On this view, we would expect evolution to proceed faster after this stage was reached than when the horns were in an incipient stage. Such is actually the case as revealed by the paleontological record. Osborn states (p. 443) that the evolutionary change in the lower Oligocene titanotheres was almost as great, while 200 feet of sediment were being deposited, as that which occurred in the Eocene titanotheres, while 2,000 feet of sediment were being deposited.

Selection for larger horns would be at the same time selection for larger body size. With the incorporation into the stock of genes having for their effect an increase in body size, the young at birth would become progressively larger and larger as the process continued. If there were no changes in the constant of growth-partition, then with the passage of time, the horn at birth would likewise become progressively larger. A size would finally be reached when the increased hazards at birth due to the larger horn would prevent successful parturition with consequently the sudden extinction of the stock. On this view the horn was first non-adaptive, it then reached an adaptive size and finally became actively unadaptive, and so became a factor leading to the extinction of the stock. Osborn favors the mechanical imperfection of the grinding teeth as the cause of the extinction of these animals. It is clear that these two suggestions regarding the extinction of the group need not be in conflict. The suggestion that the horn played a part in the extinction would appear more plausible if

it could be shown that the coefficient of growth partition for the pelvic outlet were small.

The free length of nasal/skull length relation. As the skull increases in length phylogenetically, the fronto-nasal horn progressively increases in length. Along with this increase goes an increase in the free length of the nasals in Eocene titanotheres and in Menodus among the Oligocene genera. In the other great Oligocene genera, as the horn increases in length with increasing skull length, the free length of the nasal progressively decreases, as if the horn were increasing at the expense of the nasals.

On a double logarithmic plot (Fig. 5) the free length of the nasals (y) is plotted against the basilar length of the skull (x). The specimens of each genus fall within a broad band of greater or smaller width. For the Eocene genera taken as a single group, and for Menodus, the slope of the band in each case is positive. For the other great Oligocene genera, Brontops, Allops and Brontotherium, as the skull increases in length with ascending species the free length of the nasals decreases. The nasals decrease not only relatively to the length of the skull but there is a decrease in the absolute length of the nasals. As a consequence the coefficient of phylogenetic growth-partition is negative.

In view of this result it seems necessary and desirable to make a distinction. When k is negative one might naturally refer to such a case as negative heterogony. But in negative heterogony while some phenotypic quantity (y) is increasing in absolute magnitude, it is decreasing relatively to some other increasing phenotypic quantity (x). That is, k is positive but less than 1. In the case of the characters under discussion k is negative in value and since the term negative heterogony is used in the way mentioned, such cases may very well be called hyperbolic heterogony, and stand in contrast to parabolic heterogony, which would then include both positive and negative heterogony of Huxley's terminology. Cases of

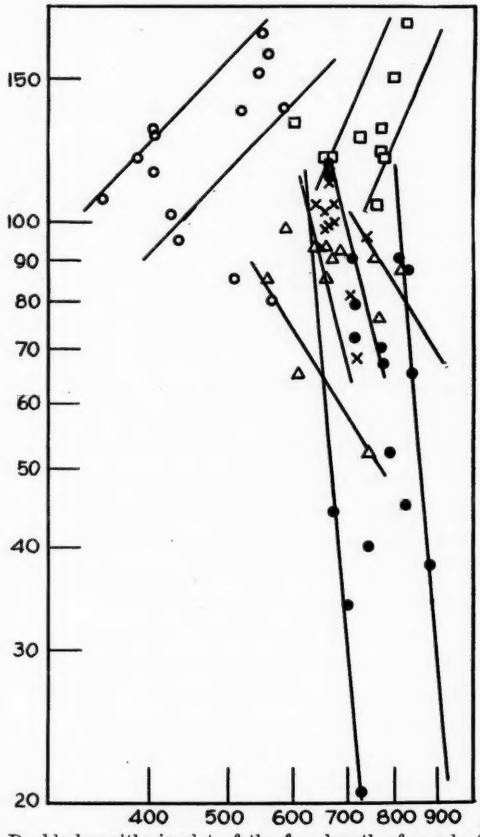

FIG. 5. Double logarithmic plot of the free length of nasals (y) against the basilar length of the skull (x). Each point represents a single specimen. Open circles, Eocene titanotheres; squares, Menodus; crosses, Allops; triangles, Brontops; solid circles Brontotherium.

hyperbolic heterogony no doubt occur during ontogeny in cases of the involution of such organs as the thymus (See Robb, 1929, Fig. 3).

It may be added here, parenthetically, that the data for applying the relative growth function to other characters yield similar results, without there being sufficient data to warrant the determination of the values of the con-

stants for the various genera separately. It is to be regretted that the difficulty in getting suitable quantitative data for the teeth, regarding the addition of new cusps and their increase in size, precludes their inclusion in the present study.

The relation between b and k. It was pointed out above, in regard to the width/length relation of the skull, that in the segment of the band representing the Eocene titanotheres, Palaeosyops occupies the upper edge of the band and Dolichorhinus the lower edge and that the edges of the band are approximately parallel. It was not intended to imply by this statement that the parallel relation is the true one and that the empirical data departed from it as a fluctuation of sampling. If the edges were truly parallel we should have two values of b for one value of k. While we know nothing which would tend to make such result seem in itself improbable, yet an examination of the relation which exists between b and k, in those instances where it is feasible to determine the relation, makes it seem quite certain that b is a one-valued function of k. More particularly, b is a decreasing exponential function of k. If such be the case, then the closely similar values of b for Palaeosyops and Dolichorhinus in the width/length relation of the skull are coincidental and no doubt dependent upon the fluctuations of sampling. As exponential relationship between the values of an ontogenetic b and k was found to hold in an investigation of the number of facets in the dorsal and ventral lobes of the eye of fifteen bar-eyed stocks of Drosophila (Hersh, 1931).

The values of b and k in the relative growth function have been determined for the zygomatic width/basilar length relation and for the $M^1 - M^3/P^1 - M^3$ relation. These values are given in Table 1. As k increases in arithmetical progression b decreases in geometrical progression, conforming to the exponential function

$$b = Be^{-nk}$$

in which b and k are the constants from the relative growth function, e is the base of the system of natural logarithms, r is the relative rate of decrease in b per unit change in k. B in the equation is the value of b when k is infinitely close to zero. The values of the constants B and r, determined from the data of table 1 by the method of averages, are respectively, 301.8 and -6.218 for the zygomatic width/basilar length relation, 141 and -5.432 for the molar/premolar-molar relation. It may be of importance for further analysis in such cases that the values of b effectively coincide when $k = 1$.

The following additional tentative rule may be added to the two given above: (3) For any two dimensions of the skull of the different genera of the titanotheres which conform to the relative growth function, b is a decreasing exponential function of k. It should perhaps be pointed out that these rules have been obtained from data on metrical characters. Meristic characters may very well follow other types of relationship.

General discussion. On the basis of the currently accepted theory of the method of evolutionary change, we may say that mutations making for larger size were the ones selected and incorporated into the titanothere germ plasm. There is no evidence to show what the mechanism was by which these genes conferred an advantage on the zygotes which contained them. It may have been by a more rapid rate of development, or by some structural or physiological character, or by some combination of such characters of the adult phase or in some other way. The reaction of the organization of the early titanotheres to these incorporated mutant genes was such as to bring about in the adults an evolutionary change which may be considered as additions to the phenotype (although there was some loss), namely increase in bulk, new cusps on the teeth, and fronto-nasal horns, all of which was accompanied by change in proportion of parts. When Brontotherium is compared with the early primitive ancestral Eotitanops, the former may be said to be decidedly more

complicated in structure. But these additions to the phenotype do not require any increase in the number of genes. All the changes may very well have been brought about by gene substitutions, mutations for increase in size displacing their allelomorphs for smaller size. In the light of genetic theory there is no good reason for concluding that there were more genes in the germ plasm of the last Brontotherium of Oligocene times than there were in the germ plasm of the ancestral Eotitanops of early Eocene times.

Since in general, among mammals, it has been found that larger animals differ from smaller ones of the same kind by the possession of a greater number of cells rather than by larger cells, we are perhaps justified in concluding that the mutant genes which were incorporated into the titanothere stock were those which brought about a greater number of cell divisions. The distribution of the evolutionary increase in the number of cells to the different homologous organs of the adult in the various species was in accordance with the relative growth function. Since the coefficient of phylogenetic growth-partition differed for different organs, the evolutionary change brought about by gene substitutions was accompanied not only by increase in the dimension of parts, but also by changes in the proportional size of parts, and their dimensions. This is clearly shown in the changed values of the various indices, but as was pointed out above comparison merely by indices may be quite misleading and confusing in any attempt to reach generalizations regarding the distribution of this evolutionary increase between homologous organs and dimensions in different species and genera. On the basis of the application of the relative growth function the general conclusions which were reached may be summed up in the tentative rules given above.

Evolution proceeds through a series of individual life histories, so that a question to which an answer is greatly desired arises, namely, what explanation in ontogenetic

terms may be given for these generalizations. Answers to such questions for any group would help to bridge the gap between genetics, embryology and phylogeny. The same general notion applies in the case of groups where fossil data are not available, that is, in general in the analysis of group variability.

While obviously in the present instance, an answer can not be given in terms of the life history of individual titanotheres, there have been recent additions to our knowledge of extant forms which might possibly apply to them. At any rate on the assumption that the knowledge, to which appeal is made below, may be perhaps of quite general validity, the writer proposes to point out some of the conclusions which follow if similar relationships existed among the titanotheres.

Painter (1928), Castle and Gregory (1929), and Gregory and Castle (1931) have shown that the large Flemish breed and the small Polish breed of rabbits produce eggs of the same size, yet the adults differ markedly in size. The adult male weights are as follows: Flemish about 6,000 gms, Polish about 1,500 gms, their reciprocal hybrids about 3,000 gms. The difference is brought about mainly by an increase in number and in rate of cell divisions. When the embryos of the large race are in approximately the 32-cell stage, the embryos of the small race consist of approxiamtely 16 cells. A difference in the rate of cell-division is maintained without difference in cell-size and without noticeable difference in rate of differentiation.

If there is no difference in the rate of differentiation, and if the larger number of cells in the larger rabbits is partitioned to the different organs and dimensions according to the same coefficient of growth-partition which prevails in the smaller rabbits, and if the weight of the organs in post-natal life may be taken as a rough measure of the number of cells, then homologous organs and dimensions from the large and the small rabbits and their hybrids should give the same value of the constants in the relative growth function.

Robb (1929) finds this to be the case for some organs. He has found, for example, that the weight of the pituitary gland and the cleaned body weight conform to the power function with no change in the values of b and k throughout post-natal life. Flemish giants, Polish dwarfs, their reciprocal hybrids, and also some rabbits of other breeds all have the same ontogenetic b and k for the partition of growth between the pituitary gland and the rest of the body. Hence it follows, if we take only the average adult weights of the pituitary gland for these several breeds and plot them in conformity with the relative growth function against the respective adult weights of the rest of the body, that the points will fall upon a straight line.

This line, on which each empirical point designates the average adult of a single breed, will coincide with the line which designates the average individual of any of the breeds throughout post-natal life. Hence, the b and k for the group coincides with the ontogenetic b and k. And so in the case of titanotheres, Rule 1 may be explained similarly in ontogenetic terms, if in their development members of different species within the same genus had the same coefficient of growth-partition (k) and the same value of b. Briefly stated and more generally, there is no change in phylogenetic b and k within the group when members of the different subgroups have the identical ontogenetic type of growth-partition. This is to be expected whenever species differ in size without any change in the type of embryonic determination. It is perhaps superfluous to point out that the converse need not be true. That is, there may be instances of no change in the group value of b and k, and yet the ontogenetic growth-partition for the average individuals of the different groups may differ.

The gene substitutions within the genus which brought about the increase in size leads in time to differences so great as to be recognized as of specific rank. The above explanation states, in brief, that these genes increased

the number of cells without change in the time relations of embryonic determination or of growth-partition during ontogeny. In such cases ontogeny strictly recapitulates phylogeny at least in regard to the ascending species within the genus.

The accumulation of other gene substitutions which were selectively incorporated into the titanothere stock brought about differences which have been designated as of generic rank. The application of the relative growth function shows that, with respect to some characters at least, genera are distinguished by changes in b and in k. Such changes may be explained also in ontogenetic terms from the general point of view of Brandt's "das typologische Grundprinzip" (Brandt, 1928).

It has been shown by an application of the method of heteroplastic transplantation of leg-buds and other organs in different species and genera of Amphibia, that at the same stage of development individuals of different species are in different states with regard to the process of embryonic determination. And the same state of embryonic determination may correspond to different developmental stages. For example, in regard to the time of determination of the anterior leg-bud, the neurula of *Triton taeniatus* is in the same state as the tail-bud stage of a species of Amblystoma. This illustrates that the date of an embryonic determination may vary in different species. Brandt (1928) shows curves which indicate that marked differences between different species in the dates of determination of leg-bud and other organs exist in this group of animals. Such relationships in the time of determination of parts he names "das typologische Grundprinzip."

While there has been (in so far as I know) no direct application of the relative growth function to data on the relative growth of the anterior leg or other organs in the species mentioned above, yet it seems clear that this difference in the relative time of embryonic determinations would entail a difference in the relative growth constants

for homologous organs in the different genera. The genera of titanotheres are distinguished from one another by differences in b and k in regard to characters previously discussed. Such differences may possibly receive explanation in ontogenetic terms in accordance with Brandt's "fundamental typological principle." More explicitly from this point of view, the character of relative growth between width and length of skull would be established at some early embryonic determination. This event is thought of as occurring at somewhat different relative times in the ontogeny of individuals belonging to different genera. And this difference brings about a change in the phylogenetic relative growth constants between genera. In other words, the difference in b and in k which distinguish the different genera of titanotheres (Rule 2) is an indication of different relative times in the ontogenetic determination of the skull dimensions. This is in contrast to the situation within the genus, where ascending species bring about no difference in b and in k (Rule 1), and which as indicated above, may be explained in ontogenetic terms on the basis of the same relative time in the ontogenetic determination of the skull dimensions.

While the embryonic determination for homologous organs in different species and genera may occur at different relative times, they obviously do not occur at random during ontogeny. An embryonic determination for a particular part may occur relatively earlier or later in regard to other embryonic determinations and other developmental processes. But there is no doubt a certain amplitude of swing between the earliest and the latest possible time for a particular embryonic determination, which may not be exceeded if the organ in question is to be produced, or if the embryo is to remain viable. Differences in b may be taken as indicative of modifications in the time of onset. While a change in the time of onset may conceivably occur without accompanying change in k (that is, b may change without change in k), yet as indicated above, the cases for which the relation between

b and k have been determined strongly suggest that for metrical characters, b is a single-valued function of k. This is true for both ontogenetic and phylogentic relative growth in the few cases for which such relations have been ascertained.

From the point of view of the "fundamental typological principle" this uniform relation between b and k (Rule 3) may possibly receive explanation along the line indicated, as an expression of orderly change in the distribution of embryonic determinations and, in the most general way, of growth-partition during the ontogeny of individuals belonging to different groups.

This application of knowledge of extant forms to the interpretation in ontogenetic terms of the tentative conclusions reached from an analysis of the titanothere data is admittedly speculative. It is intended merely to raise the question in definite form and in some detail of the relationship between ontogenetic and phylogenetic relative growth and to offer one possibility of the way in which they may be related by way of the temporal order of embryonic determination.

SUMMARY

An application of the relative growth function $y = bx^k$ to Osborn's data on skull measurements of the titanotheres shows that the function applies to phylogenetic as well as to ontogenetic growth-partition. The results may be summarized in the following tentative generalizations: (1) Ascending species within the genus bring about no change in b and k. (2) Genera are distinguished from one another by differences in b and in k. (3) b is a decreasing exponential function of k. By appealing to knowledge derived from experiments on extant forms, it is pointed out that these generalizations can be explained in ontogenetic terms.

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SHORTER ARTICLES AND DISCUSSION

PROFESSOR FISHER ON THE THEORY OF DOMINANCE

DR. FISHER'S recent note (Fisher, 1934) on the theory of dominance seems to require some comment on my part as he makes several complaints of my treatment of his work.

To begin with, he complains that "Professor Sewall Wright, who had perhaps overlooked or misunderstood the calculations in my paper, put forward some calculations of his own expressed in a different notation which for the general case gave a result identical with mine (Fisher, 1928)." If Dr. Fisher will reread the paper (Wright, 1929a) to which he refers he will find explicit recognition of agreement up to a certain point.

He also makes the following complaint: "Wright has asserted that he is discussing a case especially favorable to my views, while in fact restricting himself to the minimal postulates in its favor." I can only say that it still seems to me that a factor which in a single dose gives complete dominance of wild type would be subject to more rapid net selection than any other sort of modifier of dominance contemplated by Fisher's theory. Perhaps I should have excepted the production of superdominance, but I was not aware that this was postulated in the theory. My treatment dealt explicitly with the case of rare factors held in equilibrium by the opposing pressures of mutation and selection, the case at issue and that to which complaint No. 1 referred. No geneticist would question that dominance can often be shifted by direct selection of heterozygotes. I accepted this in my first paper (Wright, 1929a) on Fisher's theory and had in fact previously reported on such modifications of dominance in two cases in the guinea pig (albinism (Wright, 1927), white spotting (Wright, 1928)).

Again Fisher complains—and here I think with more justice—that in my recent paper (Wright, 1934) I present corrections of certain of my formulae, tracing to a correction made by him in 1929, without referring to this history. I did, however, acknowledge Dr. Fisher's correction at the time (Wright, 1929b). On rereading my recent paper, I see that I should have repeated the acknowledgment there. The correction, I may add, was too small to affect the argument against the dominance theory.

I trust that Dr. Fisher will acknowledge that he also has not always been careful in historical matters. In 1922 (Fisher, 1922) he attempted to determine the distribution of gene frequencies in certain special cases. One of his results (decay of variability under inbreeding) was inconsistent with results which I already had. On attacking the problem by a different method (involving integral instead of differential equations) I reached a general formula for the distribution of gene frequencies in populations of limited size under the simultaneous pressures of selection, mutation and migration as well as ones for simpler cases. It appeared that in the simplest case Fisher's form of distribution was correct but only through the compensatory effects of two errors. It was in this case that his rate of decay was wrong. All of his other cases were wrong. I sent my manuscript to him in 1929 in order to iron out our differences as rapidly as possible. On returning it, Dr. Fisher stated that he had found the error in his work and that he confirmed my conclusions in the first two cases. He authorized me to present this correction in my paper, which, however, owing to the saturated condition of *Genetics,* did not reach publication until 1931 (Wright, 1931). Meanwhile in his book on the "Genetical Theory of Natural Selection" and in a technical paper, both published in 1930, Dr. Fisher acknowledged my correction with respect to rate of decay of variance under inbreeding but went on to state that this had led him to a more exact examination of the whole problem. He thereupon derived corrected results for the distribution of gene frequencies in 3 special cases. There is nothing to indicate that I had obtained any results on the distribution of gene frequencies and accordingly no indication that in 2 of his 3 cases his results were merely confirmations of mine. In the third case he undoubtedly made an improvement in my formula, as given in the manuscript. The latter, while superior to his original utterly erroneous one, admittedly (Wright, 1929b) applied only to weak selection rates. I was later (Wright, 1931) able to obtain by my method the same degree of approximation not only for this special case but also in the more general cases which Fisher has never treated. The reader may compare Fisher's formulae in his publications of 1930 (Fisher, 1930a, 1930b) not only with those in my detailed presentation sent to him in 1929 but delayed in publication to 1931, but also with a general formula published in 1929 in my second paper on his

theory of dominance (Wright, 1929b). Perhaps he has overlooked this, since I find it nowhere listed in his references.

Returning to the theory of dominance, Fisher's recent paper (Fisher, 1934) goes on to expose various alleged fallacies in my reasoning. I, in turn, am unable to follow his reasoning but am willing to leave the matter at this point to the reader who wishes to compare his arguments with those which I have expressed in preceding papers or with those of J. B. S. Haldane (1930) who independently made similar criticisms of the theory. Fisher concludes with a reference to the physiological theory of dominance which I developed. "If, however, Professor Wright's views can only be made plausible by the exclusion of all alternatives he must find other objections to the selection theory more weighty than those he has revived." The reader of my paper (Wright, 1934) will find that while I contend that dominance must always have a complete immediate explanation in terms of gene physiology, I also accept selection theories as important with respect to the statistical phenomena of dominance. On pages 25 and 26, I sketched some rather obvious selective mechanisms of this sort of which I had recorded my acceptance at least as early as 1921 (Wright, 1921). On pages 50 and 51, the reader will note my acceptance of the form of selection theory advanced independently by Muller (1932) and by Plunkett (1932) as one under which dominance of wild type may increase irrespective of the past occurrence of mutants required by Fisher's theory. It was merely Fisher's particular selection theory that I excluded.

Finally in view of the tone of Dr. Fisher's note let me say here that I have the greatest admiration for his contributions to statistical genetics and biometry. If I have devoted considerable space to their criticism it is only because his views have seemed to me to be worthy of exhaustive study.

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PROBLEMS OF CROSSING-OVER IN TRIPLOID *DROSOPHILA*, WITH CRITICISM ON MATHER'S INFERENCE

(1) IN a recent issue of the *Journal of Genetics* (Vol. 27, 243–259), Mather (1933) has expressed the opinion, on the purely theoretical ground, that only two of the chromosomes of a triploid can be effectively associated at one level and that the association between the pairing units, instead of being at random with respect to the rest, tend to occur in blocks. Rhoades (1933) published a criticism on the above opinion in *Genetics*. However, he leaves the weakest point of Mather's view untouched, so that I propose to take it up briefly here.

(2) Mather's argument is based on the denial of the following two possibilities of chromosome association in a triploid, *viz.:* (i) the possibility that all the three chromosomes are effectively associated along their whole length and (ii) the possibility that only two of the three chromosomes are effectively associated at one level, but each pairing unit behaves independently of the rest. For this argument, he used the data for the long autosomes of *D. melanogaster* presented by Redfield (1930, 1932). Her study deals mainly with the diploid offspring which contain one chromosome derived from the triploid mother having one marked chromosome and two wild-type chromosomes.

Mather has given the formulae deduced from a simple expansion of the term $(7/9+2/9)^n$, which were assumed to be applicable to the foregoing systems. But, in my opinion, this assumption is incorrect. The formulae should be:

$$(0) = a + 7/9\ b + 11/18\ c + 157/324\ d + 251/648\ e + 3637/11664\ f$$
$$(1) = 2/9\ b + 6/18\ c + 122/324\ d + 246/648\ e + 4202/11664\ f$$
$$(2) = 1/18\ c + 41/324\ d + 125/648\ e + 2869/11664\ f$$
$$(3) = 4/324\ d + 24/648\ e + 812/11664\ f$$
$$(4) = 2/648\ e + 136/11664\ f$$
$$(5) = 8/11664\ f$$

(Here, a, b, c, d, etc., stand for the frequencies of non, one, two, three, etc., chiasmata, respectively.)

Substituting the numbers obtained in the experiments for the third chromosome, *i.e.*, (0) = 771, (1) = 497, (2) = 160, (3) = 20, (4) = 4, into the above-mentioned formulae and solving them directly, we get: a = 332, b = −1454, c = 3546, d = −2268, e = 1296. These values include negative quantities as well as those computed from Mather's formulae. It is, however, to be noted here that the above equations are nothing but the observed equations and that the result has been obtained by the simplest possible method. It is clear that in such a system the difference of even a single individual belonging to the classes of multiple crossovers exerts great influence on the final result. The difference may arise from various experimental errors, such as differential viability, counting mistake, reversion and mutation. But Mather treats the experimental number, especially the number of quadruple crossovers, *i.e.*, 4 out of 1,452 individuals, as the most probable one. As stated before, the values obtained by such a method do not necessarily reveal the true frequencies of chiasmata.

This problem should be carefully tested from various aspects. For example, if we take provisionally the values, as a = 0 per cent., b = 15 per cent., c = 25 per cent., d = 35 per cent., e = 20 per cent., f = 5 per cent. (the mean is 2.75 and its variance is 1.19) and substitute these values into the above-mentioned formulae, it is expected that the number of crossovers for any class may appear among the total 1,452 flies (this number is equal to the one examined by Redfield for the third chromosome) with the following frequencies: (0) = 773, (1) = 497, (2) = 158, (3) = 22, (4) = 2, (5) = 0. These calculated numbers are quite accordant with the observed numbers ($X^2 = 2.212$, $P = 0.818$), which shows clearly that both the possibilities mentioned before are never inadequate. A modified method of least squares, in which the value a is regarded as zero or nearly so, also leads to a similar conclusion.

(3) In the case where all three chromosomes are marked, the variation which is to be attributed to experimental errors other than accidental errors, may be decreased less significantly than in the case where only one chromosome is marked. The formulae fitting for the above-mentioned two possibilities may be expressed in this case by the following forms:

$$(0) = a + 2/3\ b + 4/9\ c + 8/27\ d$$
$$(1) = 1/3\ b + 4/9\ c + 12/27\ d$$
$$(2) = 1/9\ c + 6/27\ d$$
$$(3) = 1/27\ d$$

Substituting the observed numbers obtained by Redfield (1930) for the third chromosome, *i.e.*, (0) = 565, (1) = 375, (2) = 86, (3) = 4, into the above formulae and solving them directly, we get: a = 127, b = 237, c = 558, d = 108. The result includes no negative quantity and no inconsistency in the distribution of chiasma frequencies. The mean value in this case is 1.63 and its variance is 0.69, showing apparently genetical interference.

But, when the numbers obtained by Bridges and Anderson (1925) for the X-chromosome, *i.e.*, (0) = 252, (1) = 88, (2) = 23, (3) = 1, are substituted into the above-mentioned formulae, we get: a = 160, b = 24, c = 153, d = 27, which shows a considerable excess of the class of non-crossovers. This suggests strongly that the mode of synapsis and crossing-over in the triploid X-chromosomes is somewhat different from that in the triploid autosomes.

(4) After coming to the conclusion that the two possibilities mentioned before are both inadequate, Mather has tried to solve the problem from the view-point that only two chromosomes are associated at one level, and that the pairing units do not behave independently of one another. Thus, he gives two series of formulae which are based on some probable assumptions. Since I have failed to understand those formulae, I can hardly tell whether they are adequate or not. It is desirable that a more detailed analysis in the case of double crossovers, at least, are given. At any rate, he takes the observed number to be the most probable one.

Further, his assumption seems to be inadequate by the reason described below. In such a system as Mather postulated, the frequency of recurrent doubles should be greater than that of progressive doubles (*cf.* Kikkawa, 1933, pp. 42 and 43). However, Redfield obtains an equal number of recurrent and pro-

gressive doubles for any region of the third chromosome tested (*i.e.*, 63 recurrent and 69 progressive doubles; 0.71 times the probable error). Mather's explanation on this point seems dubious, for his argument is based on the conception that the two possibilities mentioned before are both absurd (*cf.* Mather, p. 256). On this point, I agree with Rhoades's (1933) criticism.

(5) Mather states that the genetical results analyzed by his method in triploid *Drosophila* agree very closely with the cytological results observed in triploid *Tulipa* and *Hyacinthus* by Darlington and his associates (Newton and Darlington, 1929; Darlington, 1929; Darlington and Mather, 1932; Stone and Mather, 1932). One of the striking features established in these monocotyledonous plants is that the mean frequency of chiasmata per one chromosome is nearly constant either in diploids or in triploids. However, even if Mather's inference be justified, the mean frequency of chiasmata per one chromosome is only about 0.69 for the third chromosome of triploid *Drosophila*, contrary to about 1.07 in diploids. This discrepancy apparently disfavors Mather's conclusion.

(6) Thus, I am inclined to the view that, as far as experimental data on *Drosophila* indicate, the three chromosomes of a triploid are all associated, if not always, along their whole length, or else that only two chromosomes are associated at one level and the pairing units behave independently of one another in the autosomes; further, that the manner of synapsis and crossing-over in the triploid autosomes is somewhat different from that in the triploid X-chromosomes. The matter mentioned last may be partially accounted for by the conception that the long autosomes of *D. melanogaster* are V-shaped chromosomes in contrast to the rod-like shape of the X-chromosome, and that there exists an essential difference in the manner of synapsis, crossing-over and disjunction between the autosome and X-chromosome. In this connection, it may be of some interest to recall the statement given by Metz (1925) from the cytological grounds on triploid *Drosophila* (see *Genetics*, 10, pp. 348–349). Mather's inference seems to be applicable only to the case of the triploid X-chromosomes. The problem of crossing-over in triploid *Drosophila* thus awaits further intensive study.

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MUTANT GENES IN A WILD POPULATION OF *DROSOPHILA OBSCURA* FALL

THE recent works of Fisher, Wright, Haldane, *et al.*, have to a large extent elaborated the theory of the genetic structure of wild populations. However, our empirical knowledge concerning the problem is still rather meager and any new data on the question, even if incomplete, present a certain interest. Especially valuable are data concerning such organisms which have been extensively studied from a genetical standpoint, as, for example, Drosophila. The works of Tschetwerikoff (1927) and of Timoféeff-Ressovsky (1927) have shown that the wild populations of *D. melanogaster* are to a very high degree "infected" with recessive mutant genes. Our preliminary results seem fully to confirm this statement as regards *D. obscura*.

During the summer of 1925, 19 females of *D. obscura* Fall were caught in a forest near Zvenigorod (district of Moscow, USSR). All of them were fecundated in nature. Already the F_1 from two of these females showed a segregation in respect to wing characters, which were designated "alae truncatae" (wings resembling "dumpy" in *D. melanogaster*) and "alae curvatae" (wings resembling "curled" in *D. melanogaster*). Subsequent analysis revealed the autosomal and recessive nature of these characters, thus proving that both the wild females studied as well as the unknown males which fecundated them were heterozygous for the factors determining these characters. Further study showed that two more of the original females were heterozygous for another gene and that a segregation took place in their progeny, though it seemed externally to be quite normal. This gene, located in the X-chromosome, had a completely sex-

limited effect, not being manifested even in homozygous females, but causing the males which carried it to produce an offspring with abnormal sex-ratios and consisting of about 96 per cent. of females and of only about 4 per cent. of males.[1]

One hundred and four F_2 cultures were raised by inbreeding the F_1 offspring. The results obtained in these F_2 cultures showed that at least six more of the initial wild flies were heterozygous for different mutant genes. The following mutant characters were obtained in the F_2:

(1) "venae plexatae" (two heterozygous wild females); veins as in "plexus" of *D. melanogaster*, but the character was less pronounced.
(2) "analis incompletus"; fifth longitudinal vein did not reach the wing margin.
(3) "abdomen abnormalis"; sclerits of the abdomen irregular, as in "Abnormal" of *D. melanogaster*.
(4) "venae incompletae"; second, fourth and fifth longitudinal veins did not reach the wing margin.
(5) "venae diffusae"; veins as in "Delta" of *D. melanogaster*, but the character was more extreme.

The mode of the appearance of these characters pointed to their autosomal and recessive nature. A further study of these flies was undertaken, as well as the analysis of their sibs. This study confirmed the correctness of the above-stated conclusion.

Except these five mutant characters, modified flies appeared in five more of the F_2 crosses, showing the following abnormalities:

(1) "venae transversae incompletae"; veins as in "cross-veinless" of *D. melanogaster*.
(2) "venae plexatae," like the above-described "alae plexatae."
(3) "alae curvatae," like the above-described "alae curvatae."
(4) "alae divergentes" (in two F_2 crosses); wings as in "Dichaete" of *D. melanogaster*.

Owing to the poor fertility of *D. obscura* under our laboratory conditions (raisin-potato-agar medium) the breeding test of these flies as well as of their sibs proved to be unsuccessful. But the fact of simultaneous appearance of the same characters in groups, *i.e.*, in several of the F_2 cultures derived from one F_1 cross, makes their hereditary nature highly probable.

Fifty-seven F_3 cultures were raised by inbreeding the F_2—progeny. Only one new mutant character was found among

[1] For a detailed description of the case see Gershenson (1928).

their offspring. This character, designated "alae maculatae," proved to be recessive and autosomal. The "alae maculatae" flies had wings darkened along the veins. As this character was found only in one of the F_3 cultures it is impossible to say whether the gene calling forth the abnormality was present already in the wild population. No new mutant characters were found in 45 of the F_4 and 43 of the F_5 crosses.

The small scale of the experiment, especially the insufficient number of the F_2 cultures raised from each of the F_1 crosses (from 3 to 8), probably prevented the detection of all the recessive mutant genes that were present in the initial flies in a heterozygous condition. Nevertheless, the results obtained show positively that the wild population of *D. obscura* is highly infected with recessive mutant genes, resembling in this respect the populations of *D. melanogaster*.

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INFECTION WITH A SINGLE COCCIDIAN OOCYST AND ITS SIGNIFICANCE[1]

TYZZER (1929) appears to have been the first to infect a host with a single sporulated coccidian oocyst, for he states that he used this means to obtain a pure infection with *Eimeria maxima* in the chicken. Jones (1932) also produced infections in chicks by feeding single oocysts. These workers employed the method of placing a single oocyst, isolated either with a micro-pipette

[1] These observations were made during experiments supported by grants from the Rockefeller Research Fund and the Industrial Science Research Fund at Iowa State College.

or by the dilution method, on a thin layer of moist gelatin, and then depositing the gelatin with the adhering oocyst far back in the pharynx of the chicken by means of a sterile forceps. In this way Jones succeeded in infecting four out of seven chicks fed single oocysts of *E. maxima.*

In a series of infections produced in laboratory rats with single sporulated oocysts of *Eimeria miyairii* the writer employed the following technique: The ends of a number of pipettes of the medicine-dropper type were drawn out in a flame to points with a capillary bore, and a number of glass microslides were sterilized in boiling water and permitted to dry. Then material from a much diluted culture of freshly sporulated oocysts was placed onto a slide a drop at a time, and examined under the low power of the compound microscope. From a drop containing one, two or three oocysts, one of the sterile pipettes with the drawn-out points was used to pick out a single oocyst, the process being observed through the microscope, and the tiny drop with but a single oocyst was deposited on another slide. If microscopic examination confirmed the presence of but a single oocyst in the tiny drop, a fresh sterile pipette was used to pick out the oocyst again. Then the oocyst with the minute amount of liquid was ejected from the capillary pipette far back onto the tongue of a previously uninfected young white rat.

Nine out of the ten inoculated rats in the first series of experiments became infected. The oocysts appeared in the rats' droppings from the eighth to the eleventh days, and always in sparse numbers. Then the oocysts from one of the nine rats thus infected were permitted to sporulate, and single oocysts from this culture were fed to each of four other previously uninfected rats. Two of these became infected. The oocysts from one of these were in turn permitted to sporulate, and two more single oocyst inoculations were attempted with this "culture." One of the two animals became infected. A single oocyst from this animal, after sporulation, was fed to a single white rat, which became infected. Then the oocysts eliminated by this last animal, which were the progeny of four generations of inbreeding of the parasite (because there is a sexual stage in the life history), were permitted to sporulate and later fed to another rat. The oocysts eliminated by this animal constitute the basis of the "pure" strain which is now being used by the writer

and his collaborators in their experimental work. Asexual stages and gametocytes and gametes of both sexes have been found in the intestinal epithelium of rats infected with this strain.

The experiment is of significance not only because it demonstrated (1) that single oocysts are capable of infecting, (2) that the parasite is capable of withstanding a certain amount of inbreeding, and (3) that it is possible to isolate a "pure" strain of this micro-organism, but also for its possible bearing on the time of the reduction division in the Coccidia. In this connection it is to be recalled that in the case of certain other Coccidia belonging to the genera Aggregata and Karyolysus, the cytological demonstrations of Dobell and Jameson (1915), Dobell (1925), and Reichenow (1921) have revealed that the reduction division takes place in the zygote after the fusion of the sexual nuclei and at the time of the first zygotic division, instead of during gametogenesis as in the case of the higher animals. Thus all asexual and sexual individuals are haploid, instead of diploid, as would be the case for all except the gametes if the reduction occurred during gametogenesis as in the case of the higher animals. The present experiment is in conformity with these cytological findings, because it does show that the zygote of the coccidium is not unisexual, *i.e.*, neither male nor female; for if it were its cleavage products would likewise be sexual and, hence, there could be no viable oocysts produced during an infection produced by means of a single oocyst, since the latter is in all cases—so far as is known—the product of the union of a microgamete and a macrogamete.

It might appear at first thought that the experiments herewith reported constitute experimental proof of the conclusions drawn from the cytological observations of Dobell and of Reichenow. Such is not necessarily the case, however, for it might as an alternative be hypothesized on the basis of the experiments alone that the zygote and the individuals proceeding immediately from it by asexual processes are diploid and hermaphroditic, like the pulmonate gastropods and the sporophytes of plants. The experiments furnish positive proof, however, that our classic conception of chromosome behavior in flies and mammals does not apply to the Coccidia. Furthermore, since a conspicu-

ous "fertilization spindle" appears preceding the first zygotic division in *Eimeria miyairii,* just as in Aggregata and in Karyolysus, it is not unlikely that the reduction division transpires at the same time in all these forms.

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INDEX

NAMES OF CONTRIBUTORS ARE PRINTED IN SMALL CAPITALS

ALLARD, H. A., Twilight Song of the Wood Pewee, 266
ALTENBURG, E., Hermaphroditism, 88; Etherizing Drosophila in Mass, 191; Artificial Production of Mutations, 491
ANDERSON, E. G., Chromosomal Interchange in Maize, 345; and I. W. CLOKEY, Chromosomes in Maize, 440
Aristogenesis, H. F. OSBORN, 193

BECKER, E. R., Infection with a Coccidian Oocyst, 571
Beetle, Lady-bird, Elytral Color Patterns of, C.-C. TAN and L. JU-CHI, 252
BOYDEN, A., Precipitins and Phylogeny in Animals, 516

Chromosomal Interchange in Maize, E. G. ANDERSON, 345
Chromosome, Numbers in Fishes, B. FRIEDMAN and M. GORDON, 446; Fragmentation, J. E. SASS, 471
Chromosomes, Somatic, of Habenaria, L. M. HUMPHREY, 184; in Maize, E. G. ANDERSON and I. W. CLOKEY, 440
Coccidian Oocyst, Infection with, E. R. BECKER, 571
COE, W. R., Sexuality in Oysters, 236
COLTON, H. S. and M. PENNYPACKER, Self-fertilization in the Pond Snail, 129
CONKLIN, E. G., The American Society of Naturalists, 385
Crossing-over and Interlocking, K. SAX, 95

DARLINGTON, C. D., Crossing-over in Drosophila, 374
DAVIS, J., Hairless Mice, 478
DOBZHANSKY, T. and J. SCHULTZ, Sex in Drosophila, 190
Dominance, Theories of, S. WRIGHT, 24; of Self-fertility in Shepherd's Purse, H. P. RILEY, 60; Mendelian, in Drosophila melanogaster, A. H. HERSH, 186; Theory of, R. A. FISHER, 370; and Professor Fisher, S. WRIGHT, 562
Dominant Mutants in Drosophila melanogaster, H. B. GLASS, 107
Drosophila melanogaster, Mutation Rate in, F. B. HANSON and F. HEYS, 97; Mutants in, H. B. GLASS, 107; Mendelian Dominance in, A. H. HERSH, 186; Sex in, T. DOBZHANSKY and J. SCHULTZ, 190; Etherizing, E. ALTENBURG, 191; Sex-linked, F. SCHUBEL, 278; Mutations in, O. JOHNSTON and A. M. WINCHESTER, 351; Translocations in, J. T. PATTERSON, W. STONE, S. BEDICHEK and M. SUCHE, 359; Crossing-over of Sex Chromosomes in, C. D. DARLINGTON, 374; Sterility in, W. G. MOORE, 508; Triploid, Problems of Crossing-over, H. KIKKAWA, 565; Mutant Genes of, S. GERSHENSON, 569
DUBININ, N. P. and B. N. SIDOROFF, Effect of a Gene, 377

EAST, E. M., The Nucleus-Plasma Problem, 289, 402
EDWARDS, T. I., Seed Frequencies, 283
Eye Defects in Rabbits, H. L. IBSEN and L. D. BUSHNELL, 92
EZHIKOV, T., Individual Variability and Dimorphism of Insects, 333

FISHER, R. A., Theory of Dominance, 370
FRIEDMAN, B. and M. GORDON, Chromosome Numbers in Fishes, 446

Gene, Effect of, N. P. DUBININ and B. N. SIDOROFF, 377
Genes, Size, C. V. GREEN, 275
Genetics Society of America, P. W. WHITING, 65, 162, 463
GERSHENSON, S., Mutant Genes of Drosophila, 569
GLASS, H. B., Eye-color Mutants in Drosophila melanogaster, 107
GOLDSCHMIDT, R., Cytoplasm and Gene-controlled Heredity, 5
Growth-curve, Autocatalytic, A. J. KAVANAGH and O. W. RICHARDS, 54

HALDANE, J. B. S., Measuring Human Linkage, 286
HANSON, F. B. and F. HEYS, Mutation Rate in Drosophila melanogaster, 97

HAUSMAN, L. A., Hair of the Sei Whale, 84; Revivification of Protozoa after Encystment, 456
Heredity, Gene-controlled, and Cytoplasm, R. GOLDSCHMIDT, 5
Hermaphroditism, E. ALTENBURG, 88
HERSH, A. H., Mendelian Dominance in Drosophila melanogaster, 186; Growth in the Titanotheres, 537
Hormonic Inhibition of a Somatic Character, P. A. ZAHL, 475
HUBBS, C. L., Racial and Individual Variation, 115
HUMPHREY, L. M., Somatic Chromosomes of Habenaria, 184

IBSEN, H. L. and L. D. BUSHNELL, Eye Defects in Rabbits, 92

JOHNSTON, O. and A. M. WINCHESTER, Reverse Mutations in Drosophila melanogaster, 351

KAVANAGH, A. J. and O. W. RICHARDS, Autocatalytic Growth-curve, 54
KIKKAWA, H., Problems of Crossing-over in Triploid Drosophila, 565

LILLIE, R. S., The Living and the Non-living, 304
Linkage, Human, Measuring, J. B. S. HALDANE, 286
Living and Non-living, R. S. LILLIE, 304

Mice, Hairless, J. DAVIS, 478
MOORE, W. G., Sterility in Drosophila melanogaster, 508
Mutant Genes of Drosophila, S. GERSHENSON, 569
Mutants in Drosophila melanogaster, H. B. GLASS, 107
Mutation, Rate in Drosophila melanogaster, F. B. HANSON and F. HEYS, 97; and Adaptation, E. J. WITZEMANN, 150; New, in Oenothera, G. H. SHULL, 481
Mutations, in Drosophila melanogaster, O. JOHNSTON and A. M. WINCHESTER, 351; Artificial Production of, E. ALTENBURG, 491

Naturalists, American Society of, E. G. CONKLIN, 385
Nucleus-Plasma Problem, E. M. EAST, 289, 402

OSBORN, H. F., Aristogenesis, 193

PATTERSON, J. T., W. STONE, S. BEDICHEK and M. SUCHE, Translocations in Drosophila, 359
Phylogeny, of Species, W. ZIMMERMANN, 381; and Precipitins in Animals, A. BOYDEN, 516
Protozoa, Revivification of, after Encystment, L. A. HAUSMAN, 456

RILEY, H. P., Self-fertility in Shepherd's Purse, 60

SASS, J. E., Chromosome Fragmentation, 471
SAX, K., Interlocking and Crossing-over, 95
SCHUBEL, F., Sex-linked Drosophila melanogaster, 278
Seed Frequencies, T. I. EDWARDS, 283
Sexuality in Oysters, W. R. COE, 236
SHULL, G. H., Oenothera mut. pollicata, a New Mutation, 481
Skull, Gorilla, Denture of, W. D. WALLIS, 179
Snail, Pond, Self-fertilization in, H. S. COLTON and M. PENNYPACKER, 129
Song, Twilight, of the Wood Pewee, H. A. ALLARD, 266
SUMNER, F. B., Taxonomic Distinctions, 137

TAN, C.-C. and L. JU-CHI, Elytral Color Patterns of the Lady-bird Beetle, 252
Taxonomic Distinctions, F. B. SUMNER, 137
Titanotheres, Growth in, A. H. HERSH, 537

Variability and Dimorphism of Insects, T. EZHIKOV, 333
Variation, Racial and Individual, C. L. HUBBS, 115

WALLIS, W. D., Denture of a Gorilla Skull, 179
Whale, Sei, Hair of the, L. A. HAUSMAN, 84
WHITING, P. W., Genetics Society of America, 65, 162, 463
WITZEMANN, E. J., Mutation and Adaptation, 150
WRIGHT, S., Theories of Dominance, 24; and Professor Fisher, 562

ZAHL, P. A., Hormonic Inhibition of a Somatic Character, 475
ZIMMERMANN, W., Phylogeny of Species, 381